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PEBRINE IN INDIA

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PEBRINE IN INDIA.

BY

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In a previous publication of this department (*Bulletin No.* 75, 1917, *of Agr. Res. Ins., Pusa*) reasons were given by the present writer for thinking that the Pasteur method of examination of moths, universally made use of in Europe for avoiding hereditary transmission of the pebrine disease of silkworms, requires modification for successful application in India. The present paper gives a more extended account of the observations upon which this opinion was based, and of the experimental data and the interpretation of them which led to the recommendation of the revised method therein described. It is hoped to publish a further paper descriptive of work on other methods of eliminating or at least of controlling this disease, depending upon placing checks upon contaminative rather than upon hereditary infection.

The Pasteur Method. This method, devised by Pasteur now fifty years ago, consists in crushing the whole body of a moth in a mortar, and examining a small fraction of the resulting powder in a drop of water under a magnification of some 600 diameters: if pebrine spores are seen the eggs of this moth are destroyed. Now it will be obvious that the reliability of such a method must depend upon the concurrence of certain factors:

(1) Easy recognition of the parasite under this magnification without staining;

(2) Sufficient number of parasites in the body of the moth to ensure their presence in the small sample examined.

The first of these requirements depends upon the presence of the parasite in the sporing condition which, as will be shown later, forms a stage in the life-history of *Nosema bombycis*, when the thick cyst wall, which affords the necessary protection to the protozoon in its resting stage outside the body of its host, also helps to give it the optical property of high refringence by which it may easily be recognized under the microscope.

The second necessary condition is one which Pasteur showed, by numerous experiments, to be accompanied by a measure of automatic control inasmuch as a small number of parasites would connote freedom from ovarian infection, *i.e.*, if the number of parasites present in the body of the moth were small enough to escape detection by microscopic examination under the conditions obtaining in Europe, then infection of the eggs would almost certainly be absent.

Now in actual practice the Pasteur method in Europe has saved the industry from the extinction which threatened it about the middle of last century, whereas after nearly twenty years of its use in India we find Professor Lefroy reporting that the principal cause of the serious decline in the silk industry in this country is the prevalence of pebrine, especially in Bengal, where the method was first introduced and adopted in the Government seed nurseries.

It was with a view to determining if possible whether the apparent failure of the method in India was due to imperfect application or to unsuitability under Indian conditions that the enquiry, of which this paper is an account, was undertaken. It may be stated at once that the results appear to show that although the method has suffered severely from ignorant misapplication and slipshod variations, yet its failure to keep the disease in sufficient control is largely due to its use without the modifications which intelligent appreciation of the great differences between European and Indian conditions would have suggested.

One of the first facts which presents itself to an investigator of this problem is the one that pebrine still persists in Europe even after fifty years' use of the Pasteur method of eliminating it. Is this due to constant survival of infection apart from that transmitted through the egg and notwithstanding the sanitary precautions and disinfection of rearing houses and seed nurseries universally adopted, or does this persistence imply a limitation in the actual effectiveness of the method itself or carelessness in its application? It is of course well known that in actual commercial practice in Europe "seed" is sold of at least two grades of freedom from infection, "cellular" seed derived from individually examined moths, and "industrial" from those of which

only a certain percentage in each batch is examined. The actual results of this method appear to be satisfactory, although it is evident that it only serves to reduce the number of cases of infection within limits which permit of commercial prosperity. It is also probable that if examination were sufficiently rigorous and the method itself sufficiently reliable, in a few years' time the disease would be practically wiped out and with it would go much of the business of suppliers of disease-free seed, as every rearer could produce his own and many of them would probably do so. In India we have an entirely different condition of affairs and it is hoped to show that by reason of such differences necessity arises for the use of seed of a higher degree of freedom from disease and consequently of the adoption of a more stringent method of examination.

The first and most obvious difference between European and Indian conditions, so far as rearing of silkworms is concerned, is the almost universal utilization in India of multivoltine races, whereas in Europe univoltines alone are reared. This fact by itself introduces two very important considerations, one directly affecting the efficacy of the Pasteur method as a means of detecting infection in the moth, and thus bearing upon hereditary transmission, and another connected with the spread of the disease by contaminative infection.

In order to gain a clear insight into the relationship of these factors with the modifications of the Pasteur method which, in the opinion of the writer, are essential to its successful use in India, it will be necessary to become acquainted with the life-history of the parasite, *Nosema bombycis*, and to obtain some idea of the mechanism of infection.

Life-history of Parasite. So many full descriptions of *Nosema bombycis* have been published by various protozoologists that it is quite unnecessary here to give more than the bare outline of its life-history as observed by the writer and to state that no apparent differences between the forms found in India and in Europe appear to occur.

The spore or resting stage consists of a thick-walled cyst the internal structure of which can only be made out with difficulty. Various stages of maturation and development of the five nuclei and polar filament may be seen in Plates I and IV. The importance of this sporogenous stage in the life-cycle lies in its power of resistance to external adverse conditions such as drought, and this power of resistance serves to carry the parasite through the period of time passed outside the body of its host and is thus responsible for transmission and spread of the disease. An important optical character of the spore is its high refringence which makes it easily recognizable in the unstained condition under comparatively low magnification (500–600)

(Plate II, fig. 1); it is this characteristic of the spore which made Pasteur's system of microscopic examination a practical possibility, as any method involving the use of such elaborate laboratory technique as is generally required for the recognition of microscopic organisms of this order of magnitude, would at once place it outside the range of practical commercial usage. Spores vary considerably in size and in general outline (Plate I) and also in staining reactions; the normal perfectly oval outline (about 1·5 μ–6 μ × 2·5 μ—3 μ) varies with the stage of maturation. A very complete description of the spore is given by Kudo (*Bull. Imp. Seri. Expt. Sta., Japan*, Vol. I, No. 1, 1916). In actual practice it is important to be able to recognize spores, perhaps one or two in a field surrounded by numerous fat globules of all sizes and shapes; it has been suggested that the removal of fat with caustic potash would facilitate this distinction but there are several serious objections to this method, not the least of which is the action of caustic alkali on the spore itself in destroying the characteristic optical refringency which is its principal distinguishing feature.

From the point of view of the "seed" producer, then, the optical character of the spore is its most important feature; to the silkworm rearer, however, its function as the resistant form of the parasite capable of securing the survival of the latter through adverse conditions of environment outside the body of its host is still more so. Every diseased worm throughout its larval period of life is shedding enormous numbers of spores mingled with its fæcal excreta, these numbers increasing rapidly with the spread of the infection in the body of the larva. It is easy to demonstrate the fact by actual experiment not only that these spores are capable of conveying the disease when first shed but that they retain their infective power for considerable periods of time even under the extreme climatic variations occurring in India. Experiments Nos. 1-14A and 1-24K (Appendix) will illustrate this point. It will be seen from the above experiments that in India the thick cyst wall and other characters of the spore enable the parasite to survive considerable variations of temperature and degrees of desiccation and the importance of this character in connection not only with the spread of the disease but its persistence will readily be understood. This aspect of the problem will be referred to later in dealing with contaminative infection and general preventive measures.

When the spore is ingested by the feeding larva, which results naturally from the contamination of the mulberry leaf on the rearing trays, either by the fæces of diseased worms or by dust containing spores derived from a similar source, the stimulating action of the digestive fluids of the gut induces

PLATE I.

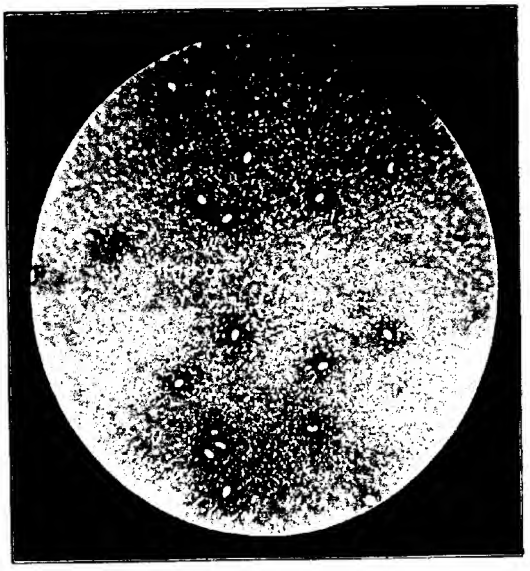


Fig. 1.

Fig. 2.

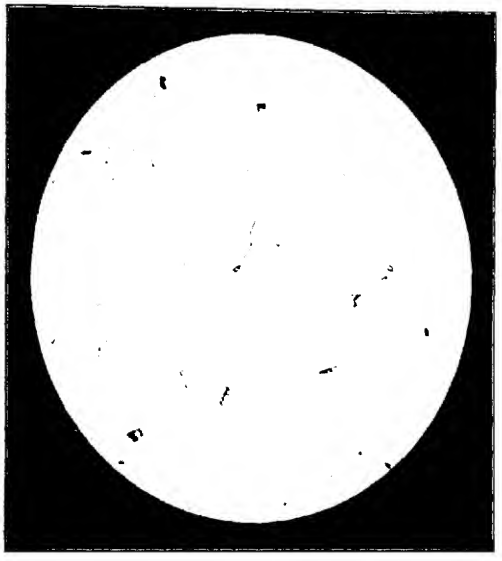
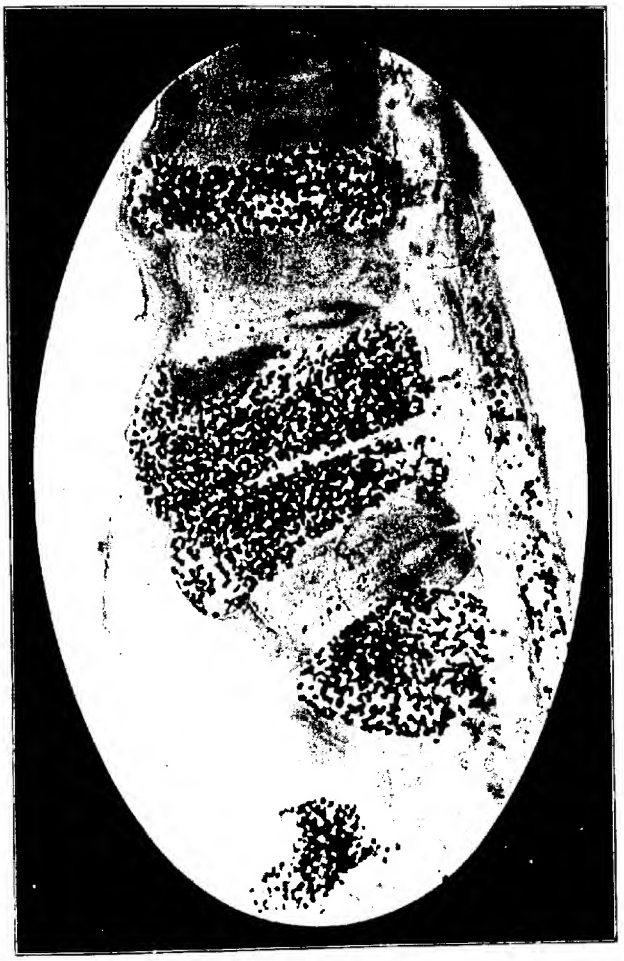


Fig. 3.

Fig. 4

PLATE III



germination. This process involves first of all the protrusion of the polar flagellum (which can also be artificially induced by the action of weak acids or iodine water) followed by emergence of the amœbula from the spore (Plate II, figs. 2, 3, 4). This motile or planont form (Stempel, *Archiv. fur Prot.*; Fantham & Porter, *Annals Trop. Med. & Parasit.*, Vol. VI, No. 2, 1912) of the parasite by reason of its amœboid character is able to penetrate between or into the epithelial cells of the mesenteron, and either remains in this situation or penetrates further into the body cavity or deeper tissues. *Nosema bombycis*, unlike *Nosema apis* which is confined to the tissues of the gut, is able to penetrate to, and multiply in, every part of the body of its host. In either case, having arrived at a suitable nidus for development, the amœbula rounds off into the meront or dividing form which has no further power of leaving the particular cell or tissue in which it finds itself at this stage (Plate III). Schizogony or merogony then commences, nuclear division and cell formation following apparently precisely similar lines and variations as those described for *Nosema apis* by Fantham and Porter (*loc. cit.*) and as shown in Plate IV. The number of meronts which can be derived by multiplication from a single one appears to be limited only by the food supply and dimensions of the particular cell in which merogony commenced: it appears extremely probable, however, that in certain tissues, such as the fat body where the walls of individual cells are relatively thin, the physiological activity of the dividing meront includes the production of hystolytic enzymes capable not only of solubilizing the contents of the host cell but of breaking down the walls sufficiently to allow of invasion of neighbouring cells by the mere mechanical pressure resulting from the rapid multiplication of the schizont. This point is of particular interest and importance in connection with the fact, ascertained by the writer by repeated experiment, that resistance to infection is very much lower in the case of larvæ in their earlier stages or "moults," as it is possible to refer this condition to the easily demonstrable relative thinness or fragility of the cell walls of practically all the tissues of the young and immature host. The practical bearing of this point upon actual rearing conditions lies in the fact that it is comparatively easy to provide specially favourable conditions both with regard to feeding and general sanitation for the young larvæ on account of the relatively small space and quantity of food required, as compared with that necessary for the full-grown, and relatively resistant, worm. In some parts of India, notably Mysore, this position has been recognized to the extent of issuing second stage larvæ from the seed-rearing stations in place of eggs, in response to popular demand based no doubt upon empirical observation. In this case, no doubt, the better results

obtained, are due to the worms passing through the susceptible early stages under such relatively sanitary and hygienic conditions obtaining in the "grainages" as are rarely found in the haphazard menage of the rearer. After passing through the stage of merogony, exhaustion of the food supply resulting from rapid multiplication in the tissues of the host results in cessation of schizogony. Each meront then becomes a "sporont" undergoing changes described for *Nosema apis* by Fantham and Porter (*loc. cit.*) resulting in the reversion once more to the spore condition.

The above very brief account of the life-cycle of *Nosema bombycis* will help to make clear the following observations on the mechanism of infection of the silkworms by this parasite under Indian conditions, and the arguments adduced in support of the use of a modified form of the Pasteur examination of moths for control of pebrine in this country.

The Pasteur Method in India. As pointed out above, the use of this method in Bengal has not been attended with any approach to the same measure of success that has been attained in Europe. It is to be determined therefore whether this failure is due to imperfections in the manner of applying the method or to inherent unsuitability of the latter to Indian conditions, or to a combination of both causes.

As before stated, the European method consists in crushing the whole body of the moth in a mortar and examining a small portion of the resulting powder in a drop of water under the microscope. On visiting various seed nurseries in Bengal where examination was going on it was found that in a large number of cases the use of a mortar and pestle was dispensed with altogether, their place being taken by a small scrap of paper, generally newspaper, which was folded over the abdomen of the moth, the latter being then squeezed so as to expel some of the body fluids on to the fold of the paper. A drop of this fluid was then placed on a slide for examination. It was explained that this procedure was less troublesome than that involved in the use of a mortar and pestle which had to be washed after every examination, and that it moreover saved time and the money which would have to be spent on the numerous mortars required in a grainage of any size, whereas the paper could be thrown away. Now, very little consideration or examination of this most reprehensible method, by anyone conversant with either the anatomy of the moth or the infective power of pebrine spores, would be sufficient to condemn it utterly as a dangerous and entirely inefficient imitation of the original one. In the first place, the result of compressing the abdomen of a moth in the manner described is merely to discharge the fluid contents of the colon (Plate VI) which consist mainly of a watery suspension of particles of

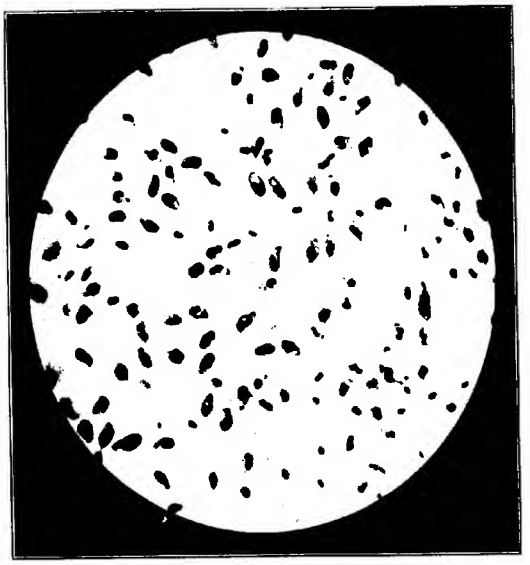


Fig. 1.

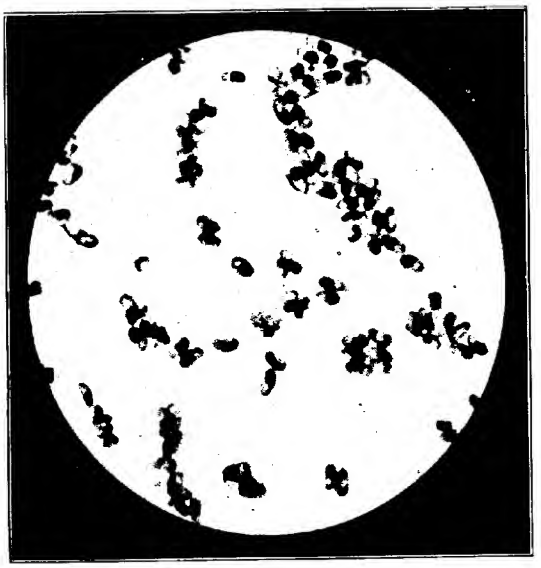


Fig. 2.

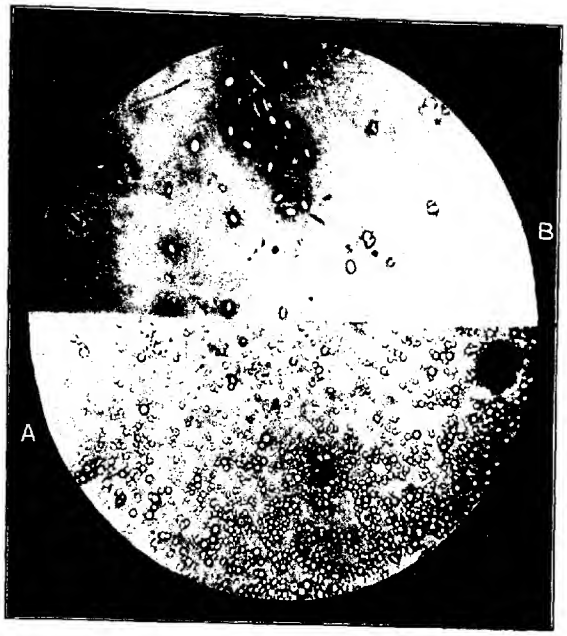


Fig. 3.

Fig. 4.

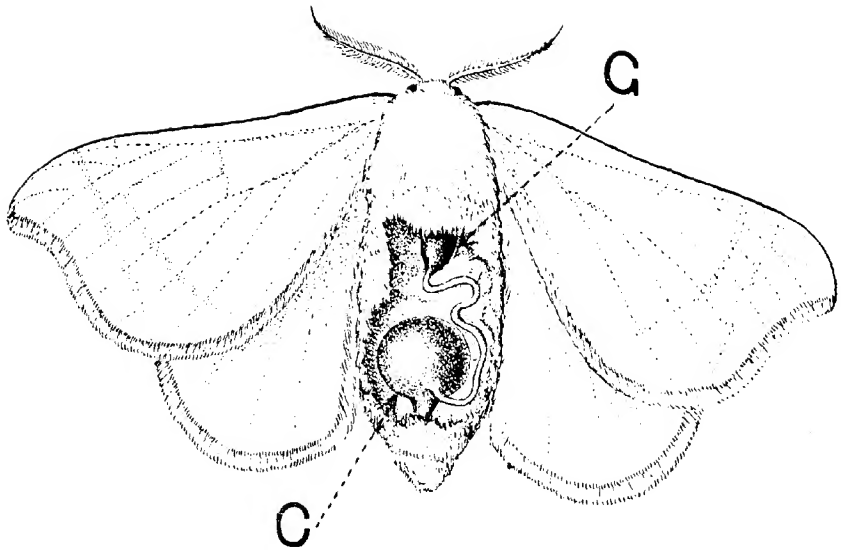


Fig. 1.

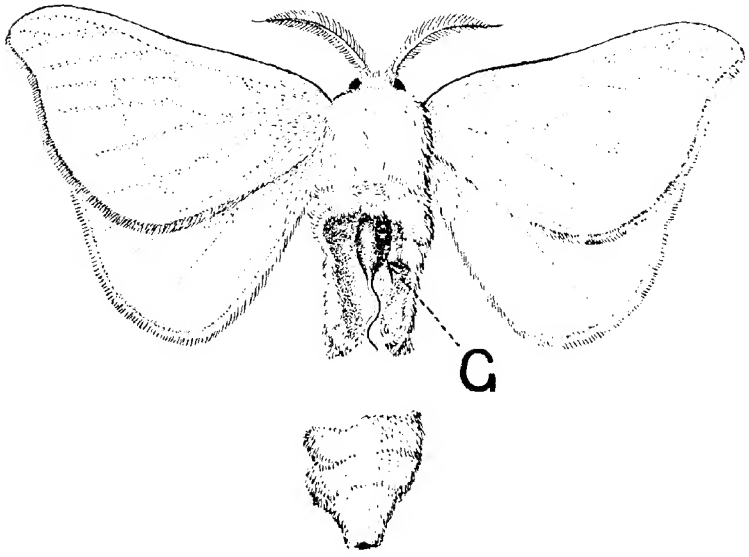


Fig. 2.
Gut (G) and Colon (C) of Moth.

fat (Plate V, fig. 3A) * and are most unlikely to include any pebrine spores present in the body tissues except in cases of comparatively heavy infection which alone will be detected by this procedure. Secondly, the use of this paper method not only fails to detect a large percentage of infected cases but the paper after use is liable to fall on the ground and be kicked or blown about, and if utilized on a heavily infected moth will be saturated with pebrine spores which will be distributed on drying up so as to infect the whole examination room, and many otherwise disease-free layings brought along with the moths under examination. This danger also arises in connection with defective technique in examination rooms which permits of the drying up of smears on slides and escape of spores as dust before immersion in the antiseptic bath, generally, but not always, provided to prevent this. The writer has seldom failed to detect pebrine spores in the floor dust from examination rooms in India even in cases where these floors were of cement.

Users of the paper method claimed as an advantage the possibility in practice of examining 1,200 moths per microscope per day ; now, allowing for eight hours of actual work this is at the rate of one moth per 24 seconds ; it is perhaps unnecessary to discuss the reliability of such microscopic examinations carried out at this pace.

When the proper mortar and pestle method is used the chances of recognizing the presence of pebrine in the body of a moth depends upon the truly representative nature of the sample drawn for examination ; in cases where the amount of infection is comparatively small, the uneven distribution of spores throughout the tissues and the varying resistance of the latter to disruption and comminution diminishes the chances of obtaining a good sample, which can only be got as the result of prolonged and careful grinding. It is largely for this reason that the important differences between the univoltine races of Europe and the multivoltine in India have to be taken into consideration. In Europe a period of several months elapses between oviposition and hatching of the eggs ; this allows examination of the moths to be undertaken at any convenient time, generally several weeks after the laying of the eggs ; during this interval the dead body of the moth has undergone natural desiccation so that when crushed in the mortar it is easily comminuted and a good sample obtained. In India, however, in the case of multivoltine races the

* Plate V, fig. 3 shows the appearances found by the ordinary microscopic method of examination of material from the crushed whole moth (*a*) and from the gut of the same specimen (*b*) respectively. This moth would have been passed as disease-free by the Bengal method.

eggs hatch out within about eight days of oviposition, during which period the whole of the moths must be examined; this results, even where mortars are used, in relatively imperfect sampling owing to the difficulty of properly grinding the fresh body of the moth containing as it does not only body fluids but the liquid contents of the colon above referred to. Actual experiment has shown that in the case of lightly infected moths, which, however, had produced diseased eggs, prolonged grinding in the mortar was required to yield obviously infected samples for examination. This fact would necessitate a very considerable multiplication of the examining staff if reliable results are to be obtained except at a very reduced rate. Artificial drying of the moth, although facilitating comminution, does not entirely remove this difficulty for reasons about to be given.

A second and still more important consideration arises out of the greatly diminished period of time between oviposition and hatching in the case of the multivoltine races. It is a matter of very general knowledge derived from the experience of seed rearers in Bengal, that the chances of recognizing pebrine in the moth increase very considerably with the period of time elapsing between oviposition and examination, and in fact the latter is delayed as long as is practically possible for this reason. A natural explanation of this phenomenon would seem to be afforded by the assumption that the parasite continued to increase in numbers in the body of the moth even after death, but acquaintance with the life-history of *Nosema* and examination of sections affords an equally simple elucidation based on actual observations. As described above, the parasite in any particular tissue or cell of its host multiplies by merogony, this condition of activity persisting until the food supply available is exhausted when sporogony ensues. Now in the body of a diseased moth at the time of oviposition a very considerable percentage of the parasites may still be present as meronts, and, as such, entirely unrecognizable by the ordinary microscopic examination without staining (Plate V, fig. 4). As the body dried up after death the consequent concentration of the cell contents of the infected tissues would inevitably render further merogony impossible, with the result that sporogony would take place. The extent to which meronts would be replaced by spores would no doubt depend upon the percentage of the former which had arrived at a sufficient stage of maturity to enter upon this next stage of the life-cycle, but examination of sections suffices to show that rapid desiccation, as might be expected, will inhibit this change, so that artificial drying of the moths, although facilitating the process of trituration, will not increase the number of spores present, whereas this increase does actually take place when desiccation proceeds at the natural rate.

Here then we have a simple explanation, based on the relative visibilities of the two forms of the parasite, of the great disadvantages attending the use of the Pasteur method when applied to the multivoltine races of silkworms in India. It is easily demonstrable that where infection has occurred late in the life of the caterpillar, the moth at the time of examination, a few days after oviposition, may contain numerous merouts which, being practically unrecognizable in the unstained condition, would afford no indication of disease to the ordinary examination, whereas the number of spores present may be so small as to escape detection. Reasons will be given in the section of this paper dealing more particularly with hereditary infection, for thinking that this infection may result from the presence of a comparatively small number of parasites in the body of the host, so that if the percentage of spores to merouts is low, the former may escape detection even although ovarian infection has taken place.

Another important point bearing upon the elimination of the disease in India arises out of the use of multivoltine races. Owing to the succession of broods throughout the year, not only is the amount of infective material produced, taking the form of spores derived from faecal excreta, much greater than is the case with the univoltine races, but the opportunities for removing or destroying it, either by antiseptic measures or the operation of time, are proportionately much less. For the same reason an initially insignificantly small percentage of diseased larvae will serve to convey infection by contamination during the season to a disproportionately large number out of the succeeding broods reared in the same house, this increasing amount of infection being again made greater by the generally insanitary conditions obtaining in the rearing houses in this country. For this reason it is necessary in India to aim at a much more rigid exclusion of hereditary infection than is required in Europe, owing to the cumulative effect of any initial disease upon subsequent broods. It will appear from the foregoing considerations, therefore, that although the failure of the Pasteur method in India may have been largely due to the seriously defective manner of its use in the past in this country, yet in view of the greatly different conditions resulting mainly from the very general use of multivoltine races and partly from unhygienic surroundings, it would seem necessary to consider the possibility of introducing some modified method of applying it. Any such modified system of examination must not only be based upon recognition of the considerations arising out of the use of multivoltine races, but must aim at a more rigid standard of exclusion of disease for the reasons given above.

The Revised Method. This depends simply upon localizing the seat or centre of infection and concentrating examination upon this point; this method would depend upon whether the natural course of infection tended to produce such a focus and to do so in such a manner as to render the latter readily recognizable and easy to examine. Study of the mechanism of infection in the silkworm has led the writer to conclude that if pebrine infection is present at all in the moth it will be found in the gut, or chyle stomach, either in the contents or in the wall of the latter, but very generally in both. Examination of hundreds of specimens supports the view that the exceptions to this rule are of the order of less than one-tenth per cent. of all classes of diseased specimens, whereas, in the case of light infection, as many as fifty per cent. may escape detection by the mortar and pestle method as used within the restricted period for examination of multivoltine races in India. The origin of the exceptions referred to is due to the remote possibility of hereditary infection carrying on through one complete generation without invasion of the alimentary canal, but the survival of the individual and the absence of infection in the gut under these conditions is so improbable as to place this class of cases outside the range of practical consideration. The revised method of examination consists in separation of the lower portion of the abdomen, thus exposing the dark coloured and easily recognizable midgut (Plate VI). A small portion of this organ removed with a needle and rubbed with water on a slide will show the presence of pebrine spores if they occur in the body of the moth in sufficient number to be detected by rough microscopic examination; the considerations leading to its adoption may now be set forth in more detail than was given in the previous paper describing this method.

Experimental. The experimental methods by which the following results were obtained depended largely upon the proper isolation of the various lots of larvæ under different treatment. This was secured by the use of wire-gauze rearing cages supported by cross wires over trays of water. The use of the latter prevented the drying up of the fæces and their dissemination as dust and with this dust any pebrine spores they might contain. Diseased larvæ, whether naturally or artificially infected, were reared in a separate room from the healthy lots. Complete disinfection was made possible by the use of the wire cages and stone floors and walls. It may be mentioned here that experiment showed the almost complete inefficacy of copper sulphate solutions of the strength generally used for sterilizing rearing houses and appliances in Bengal, whereas formaldehyde, either as vapour or sprayed at 1 per cent. strength, completely destroyed the infective power of fresh pebrine spores.

Fig. 1.

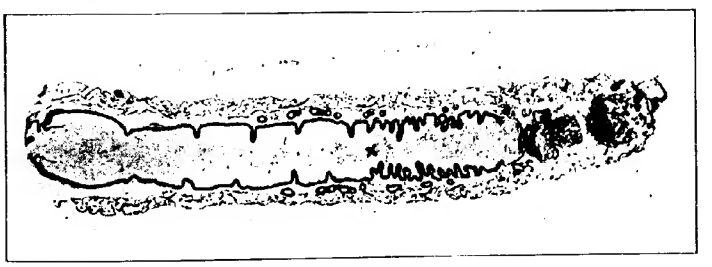


Fig. 2.

Artificial infection was carried out by feeding larvæ on leaf smeared with water suspensions of infected material derived from diseased pupæ or moths, examined microscopically to determine the presence and number of spores; this method did not of course permit of more than an approximation to quantitative conditions so far as the amount of inoculum and its virulence were concerned but by the use of large numbers of worms both under treatment and as controls, and by taking the latter from the same laying as the former and multiplying the number of experiments, convincing results were obtained in most cases.

Examination of diseased specimens was carried out both by dissection, and by direct smears, but mainly by microtome sectioning. A large amount of preliminary experimental work was carried out to determine the most appropriate methods of fixing and staining. The successful production of sections suitable for photographic record and showing the parasite in the tissues required the employment of special fixing and staining methods, the most successful of which for general sections involved the use of either Carnoy or Schaudinn fixatives and of regressive staining with Hæmatoxylin and Ferric Alum, followed by counterstaining with either Erythrosin or Eosin and Methylene Blue. For differentiation of spores and meronts in tissue sections a regressive method involving overstaining with Iron Hæmatoxylin followed by decolorization with Ferric Alum and counterstaining with Eosin and Methylene Blue gave slides in which the spores retained the Hæmatoxylin whilst the dividing forms showed differential staining (Plate V, fig. 4; and Plate VII, fig. 1). This method of course required careful watching during the decolorizing stage. This method of staining was also useful for free spores (Plate IV) although liable to produce shrinkage if pushed too rapidly. Giemsa, being almost unobtainable (1917) and of uncertain action,* was but little used; this lack along with that of orthochromatic plates constituted a serious difficulty in dealing with the life-history of the parasite.

It was fortunately found that Ilford ordinary plates are sensitive to light transmitted by Wrattens B filter (M series); this allowed fairly good photographs of thin sections stained with Hæmatoxylin and Erythrosin to be obtained, but smears showing meronts and spores in various stages of maturity stained with Giemsa or Leishman could only be photographed on panchromatic plates which were of unknown antiquity and considerably lowered the quality of the results.

* The only Giemsa stain available had been in solution for an unknown number of years; the dilute solution had to be used within a few minutes of making up otherwise nuclear staining generally failed altogether.

In every case parallel sections or preparations of normal and diseased subjects of similar age and which had undergone similar treatment, were utilized in order to minimize errors of interpretation ; in this way a complete duplicate set of normal and diseased sections of all the tissues of the larva, pupa and moth, was obtained ; sections of eggs were also included, making some 800 slides in all.

The Mechanism of Infection. Infection originates through ingestion of food contaminated with pebrine spores ; this general proposition only requires modification to the extent of admitting the possibility of wound infection described by Pasteur as resulting from introduction of spores through small punctures in the skin of the worms caused by the spiny protuberances on the hinder segments of other worms crawling over them on the feeding tray ; although this mode of infection may be possible the experiment upon which Pasteur based his conclusion was not carried out under conditions which excluded the possibility of the small percentage of infection observed having origin through ingestion of spores with the food, these spores being derived from stray portions of the inoculum used, which was merely introduced on a needle inserted into the skin. It is difficult to understand, moreover, how spores introduced in this manner by extremely shallow punctures, would find conditions suitable for germination. Numerous experiments made at Pusa failed to obtain any positive results with this method, and although such failure in India might be accounted for by climatic differences, it seems clear that for practical purposes, where the same climatic factor would operate, this mode of infection may be considered of negligible importance.

Every feeding worm consumes about half its own body weight of leaf at each feed of which some half a dozen are given in the 24 hours, the actual amount of leaf varying with the age of the caterpillar. A third stage larva, *i.e.*, about half grown, consumes about 0·05 gm. of leaf at a meal and this represents some 187·5 sq. mm. of leaf surface reckoning only one side of the leaf. In a rearing house where infection of any severity exists, pebrine spores can be found on every exposed surface, and even in a previously disinfected house, the presence of individual infected worms on a rearing tray will rapidly give rise to spores, derived from their faeces, which very soon will become dispersed not only over the leaf and the tray in their immediate neighbourhood but over other trays surrounding or below the original one. The chances of ingestion of spores by any one worm will depend then largely upon the extent of the surface of leaf consumed, but the actual chances of such ingestion, taking all the worms on a tray, *cæteris paribus*, will depend upon the concentration of worms per unit of surface, so long as the whole of the leaf is not

consumed. In actual practice in India only about half of the leaf is eaten, the remainder generally drying up and becoming unpalatable before this occurs. It may be said therefore that in general, where infective spores are present, the chances of infection by ingestion of such spores increases in proportion to the number of worms per unit of feeding area, *i.e.*, in proportion to the overcrowding of the caterpillars on the trays. The result of overcrowding in raising the incidence of pebrine infection is well known, but is generally attributed to the lowering of resistance consequent on the unhygienic effect of such crowding and of the competition for food associated therewith.*

The spores ingested pass with the food into the mesenteron or midgut; here the chitinous lining of the anterior portion of the alimentary tract ceases and is replaced by an epithelial one which, according to Gehuchten and others, provides for the carrying out of the functions both of digestive secretion and absorption. Actual infection of the host depends first upon germination of the ingested spores and, according to Stempel (*loc. cit.*), this is brought about by the salivary excretion and not by that originating in the midgut; this view is not held by other observers (such as Zander 1911 and Kudo 1913), nor do they agree with the correlated opinion that auto-infection, *i.e.*, fresh infection arising from spores actually originating in the same host, does not occur in the silkworm. If germination of the spore depends upon its activation by the salivary secretion and cannot result from the stimulation of any other body fluid, then it appears probable that Stempel's dictum on this latter point would be correct. Kudo (*loc. cit.*) does not accept this view but believes germination to depend upon the digestive juices of the midgut; this theory would allow of the germination of spores formed in the gut epithelium and cast out into the lumen, such germination giving rise to fresh infection (auto-infection) in the same host. So far as the writer's observations bear upon this subject they lead to the following conclusions: (1) That germination of pebrine spores probably takes place more readily in the acid medium found

* It is very probable that one of the effects of overcrowding is the consumption of the dried up leaf, or rather of portions of it which would ordinarily be left untouched owing to the natural instinct of the caterpillars leading them to avoid unsuitable diet. Such withered and partly dried leaf is evidently prejudicial to the health of the caterpillar either by reason of changes in the leaf tissue due merely to loss of moisture, or to more complicated ones associated with respiration or the action of intracellular enzymes. Thus artificially induced hunger, due to overcrowding and consequent competition for food, will to some extent overcome the natural instinct which protects the silkworm from consuming unsuitable food. This point is of interest as helping to explain the well-known sensitiveness of the silkworm to unsuitable conditions of rearing, and the difficulty experienced in carrying out this operation on a factory, rather than on a cottage scale.

in the lower half of the midgut than in the alkaline secretions of the proventriculus and upper end of the former : (2) that germination is not confined to the alimentary tract but may take place in other situations as the result of the stimulating action of enzymes or ferments connected with metabolic processes other than digestion ; this point will be dealt with later in discussing hereditary infection, : (3) that auto-infection will depend largely upon the original infection of the larva having taken place early enough to permit of maturation of the spores from which such auto-infection is to arise. As has been found by other observers (Stempel, Kudo, *loc. cit.*), several days' time is required for the completion of sporogony, and it will be seen on examination of Plate I, that maturity of the spore as indicated by protrusion of the flagellum is arrived at by successive stages, before the completion of which germination cannot take place ; hence auto-infection implies the lapse of sufficient time for the complete life-cycle of the parasite, including the maturation of the spores, between the original infection and the secondary one ; in the case of hereditary disease auto-infection might occur in the early stages of larval life, but where this has resulted from contaminative infection through spores ingested with the food, secondary infection is not likely to be found until a much later period.

Germination of the spore results in the protrusion of the polar filament (Plate II, fig. 2) and the emergence of the amœbula ; this is a minute oblong irregular shaped mass of protoplasm, containing two nuclei which either unite to form the uninucleate " planont " described by Stempel as subsequently multiplying by division, or remain as nuclei of daughter cells arising from direct division of the parent amœbula (Kudo). The writer was unable to decide which of these methods or whether both of them appear in the Indian silkworm. In the case of *Nosema apis* Fantham and Porter (*loc. cit.*) have already described the entry of the amœbula into the epithelial cells of the gut of the bee, and although the writer has not been able to observe this penetration actually proceeding, it is easy to confirm the occurrence of this process by consideration of serial sections of artificially infected larvæ.

As the infecting spores enter the gut mingled with the food they will naturally be carried for some distance along its length by the peristaltic current before germination can take place, if this occurs as a result of the action of the digestive secretions of the midgut ; if, however, as Stempel supposes, such germination actually takes place only in the foregut, then we should expect to find infection most pronounced in the fore-end of the midgut and falling off in intensity towards the hinder part. This, however, is not the case where auto-infection has been prevented by examination of larvæ at short intervals

Fig. 1.

Fig. 2.

of time after artificial infection; Plate VII. fig. 2 shows the point in the midgut at which such infection first occurs, this point invariably being some distance from the opening into the proventriculus, and suggesting by its position not only that germination takes place first in the midgut, but that the point of earliest infection of the epithelial cells of the gut wall is partly determined by the translational effect of the peristaltic current. This latter factor is of importance in considering the question of resistance to infection. It is a well-established fact, easily confirmed by experiment, that such resistance is higher in the case of well-fed larvæ kept under hygienic conditions; such worms eat largely and rapidly, and the peristaltic current and passage of fæces are regular. No doubt there are many other possibly preponderant causes underlying natural resistance, but it seems clear that the peristaltic current plays a part in reducing the surface of the gut actually exposed to penetration and thus lessens the total amount of infection.

Examination of gut sections of recently infected worms shows, as would be expected, that in the majority of cases infection first takes place at the bottom of folds of the gut (Plate VIII. fig. 1), where the germinating spore and the emergent amœbula have escaped into a backwater of the peristaltic current and there, no doubt helped by the protruded flagellum, have remained in contact with the wall of the gut long enough to allow of penetration. It must be remembered that the amœbula, set free amongst the food in the lumen of the gut, has first to penetrate the peritrophic membrane, which, being a chitinous tissue, might be supposed to afford considerable resistance to its passage; there does not appear, however, to be any evidence in favour of the supposition that resistance to infection implies any connection between a healthy condition of the host and more perfect development of this membrane either in the direction of complete continuity accompanied by freedom from rupture, or greater thickness. With regard to the first point, examination of numerous specimens showed that in diseased (pebrinised) larvæ there is a decided tendency to imperfection in the peritrophic membrane possibly due to the effect of the disease upon the secreting cells of the proventriculus in which it originates; such imperfections may take the form of actual solutions of continuity in the envelope itself, or, in milder cases, of unequal thickness predisposing to rupture. The principal function of the peritrophic membrane would appear to be the protection of the epithelial lining cells of the midgut from any disruptive mechanical action due to contact with hard portions of the food (such as fragments of midrib of the leaf), during the powerful muscular contractions of the gut wall incidental to the processes of feeding and defæcation. Considering the extreme tenuity of the membrane it is remarkable

how free from ruptures the majority of those examined coming from healthy worms was found to be, when tested in water by inflation with air from a small glass inflator. On the other hand this was not the case with diseased worms although it has not yet been ascertained whether this was caused by imperfect original formation or subsequent deterioration ; the resulting condition however is an effect and not a cause of infection ; its bearing upon the spread of the disease to other hosts is important and will be discussed later.

Any increase in the thickness of the peritrophic membrane might be considered beneficial as likely to heighten resistance to penetration by the parasite, but the only instances within the writer's experience of such thickening have been associated with a diseased condition resulting from bacterial infection ; these instances occurred in "Eri" (castor-oil plant) silkworms (*Attacus ricini*) suffering from an intestinal complaint resembling "flacherie" due to bacterial fermentation accompanied by great thickening of the peritrophic membrane ; in many cases this membrane was invaded by the intestinal bacteria which did not however appear to enter the cells of the gut wall. Plate VIII, fig. 2 shows such invasion accompanied by enormous thickening of the membrane ; the result of this thickening was very evident in every one of some hundreds of sections examined. Without any exception an apparently entire absence of digestion of the leaf (in this case castor— *Ricinus*) in the gut was observed ; this could be inferred not only from the unaffected condition of the fragments of leaf (Plate IX, fig. 1), but from the absence of the viscous fluid resulting from the action of the digestive ferments upon these fragments which is a characteristic feature of the spaces between those latter in the healthy gut (Plate IX, fig. 2). It is clear that this condition is due in part to the obstruction offered by the thickened peritrophic membrane to the intermingling of the digestive secretions of the epithelial cells with the food in the lumen of the gut. No doubt the acid reaction frequently associated with the bacterial fermentation of the food characteristic of this disease would seriously interfere with the action of the digestive ferments (Sawamura, *Bul. Col. Agr. Tokyo*, Vol. IV). It is not clear how far this interference with digestion is responsible for the death of the affected worms but it seems evident that this very marked feature of the disease differentiates it as a bacterial infection from other cases of "flacherie" in which no such condition seems to have been observed. In any event it will be obvious that any thickening of the peritrophic membrane must inevitably interfere with the normal processes of digestion and for this reason could not have any favourable influence even if tending towards prevention of invasion of the epithelium of the mesenteron by the pebrine parasite. Some variation in

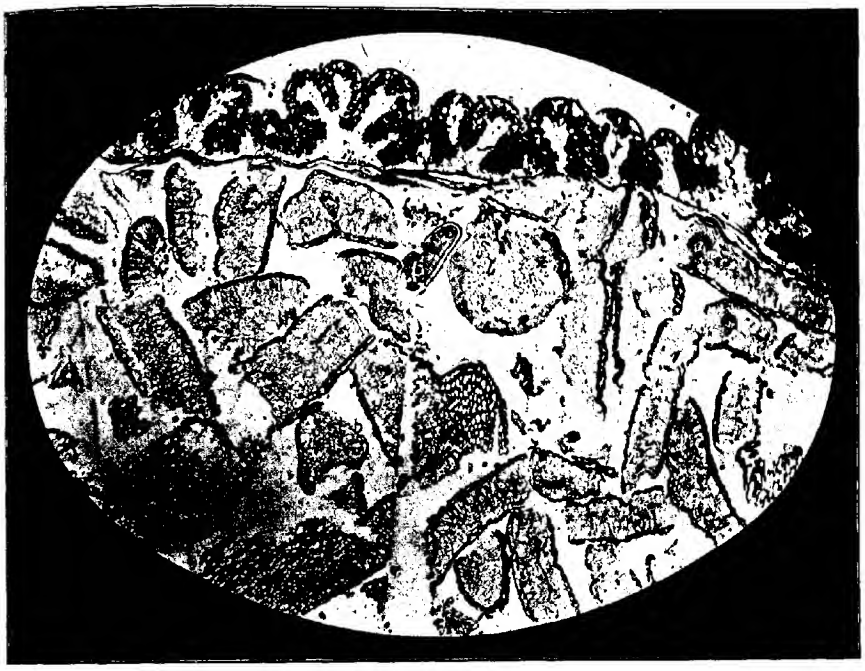


Fig. 1.

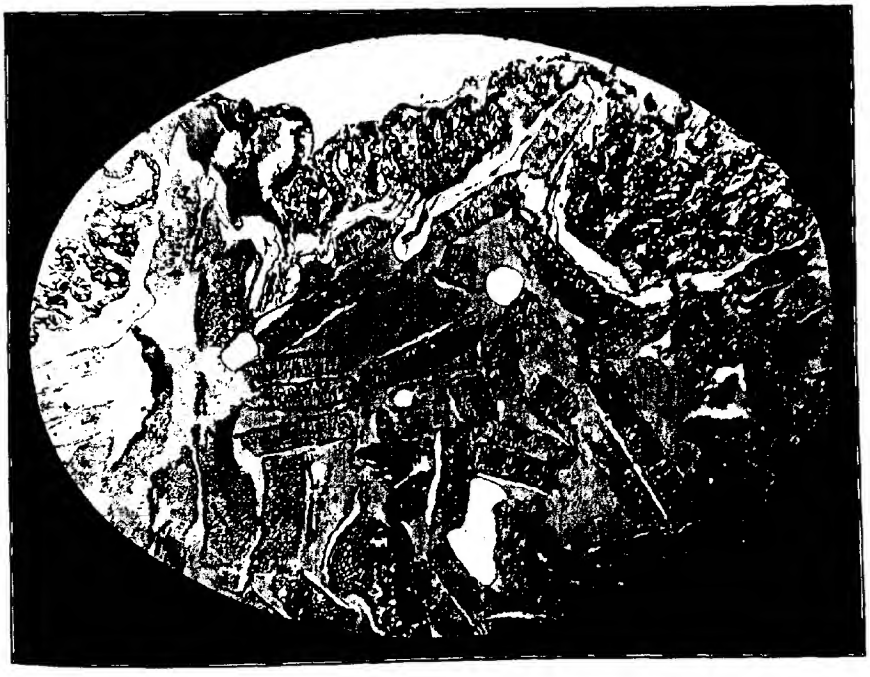


Fig. 2.

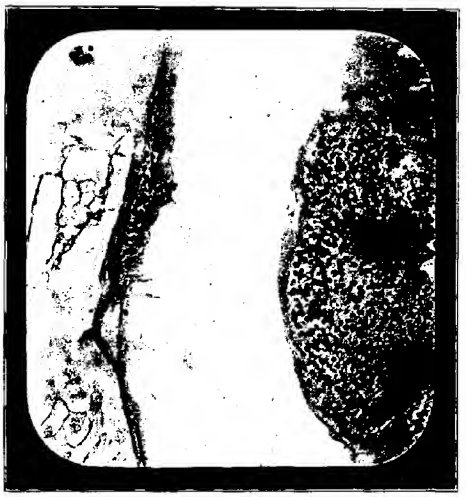


Fig. 1

Fig. 2.

Fig. 3

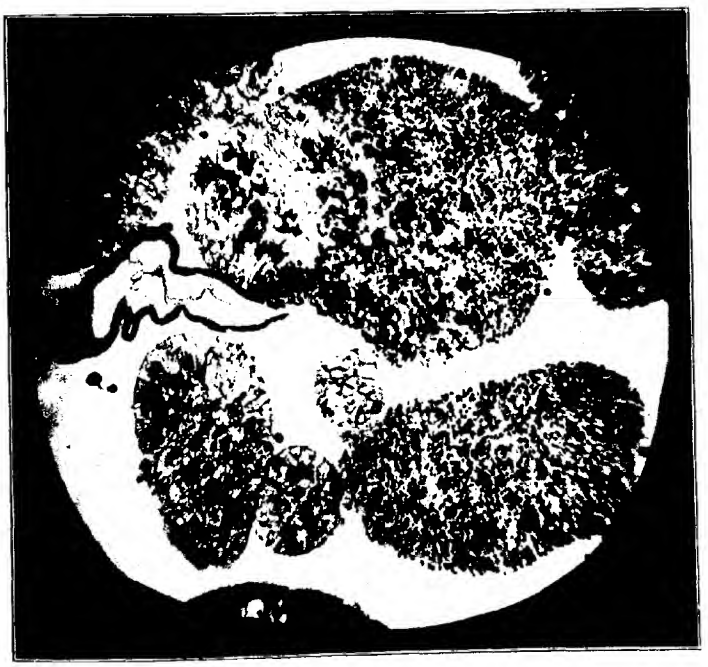


Fig. 4

Fig. 1.

Fig. 2.

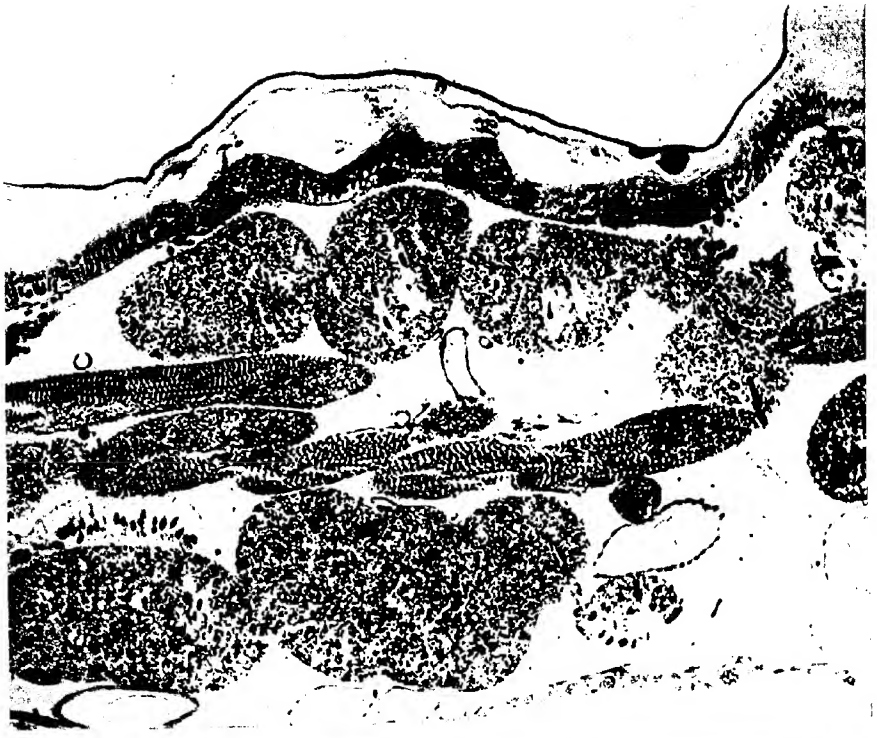


Fig. 1.

Fig. 2.

thickness does undoubtedly occur but this is only apparent, being due to deposition of mucus on the side next to the epithelial cells by excretion from the latter; this deposit can be distinguished from the original membrane by two observed facts: (1) the actual occurrence of invasion of the mucous layer by the pebrine parasite followed by merogony therein, which has not been observed by the writer in any chitinous tissue (Plate X, fig. 1); (2) by differential staining with Cotton Blue which easily stains chitinous tissues only penetrated with difficulty by ordinary stains. The very great thickening of the peritrophic membrane in the diseased "Eri" worm above described, seems to be caused in this manner as a result of the stimulation of the epithelium by bacterial toxins formed in the gut, and as the membrane is brought into close contact with the wall of the gut by the pressure of the food contained in it, there would appear to be no difficulty in this assumption.

The amœbula having passed through the peritrophic membrane penetrates into the epithelial cells of the wall of the gut. This movement carries the planont to varying distances so that in some cases it comes to rest in the secretory epithelium of the gut wall (Plate XI, fig. 1), whilst in others it is found to have penetrated as far as the body cavity where it may enter other organs such as the muscles, fat bodies, silk gland or even the more distant hypodermal cells just below the cuticle itself (Plate XII, fig. 1). At a later stage of the disease, or where this is due to hereditary infection, every tissue of the body may become infected; Fantham and Porter (*loc. cit.*) have pointed out the inferior deadliness in this respect of *Nosema apis* the growth of which is almost entirely confined to the gut although the planont is capable of penetration into the hæmocœlic fluids of the body of the bee. In the case of *Nosema bombycis* the mode of penetration seems to indicate a centrifugal rectilinear movement varying in extent possibly with individual planonts or with the resistant character of the invaded tissues, but not suggesting any chemotactic influence in determining its direction or termination so much as a varying supply of initial energy. On an average this supply would appear to carry the majority of planonts about as far as the ends of the epithelial cells furthest from the lumen of the gut; here infection first becomes obvious (Plate X, fig. 3) four days after artificial infection by feeding; one or two days later infection appears in the two muscular layers, circular and longitudinal, surrounding the gut and very shortly after this in adjacent organs such as the silk gland; Plate XII, fig. 2 illustrates very well the orientation of infection with reference to its point of origin in the gut; it will be seen that infection has taken place only on that side of the silk gland adjacent to the gut. The later appearance of infection in other tissues is therefore

probably due merely to the smaller number of more active planonts which reach these more distant situations, and become evident only when merogony has multiplied them to a recognizable number. It is a noticeable feature of infection in younger larvæ that this appears to spread more rapidly and to more tissues than is the case in caterpillars of more advanced stages of growth. This is a point of some importance in consequence of its bearing upon the relative resistance to infection of older as compared with younger larvæ; it does not seem necessary to assume that the greater power of diffuse infiltration possessed by the parasite in the latter is entirely due to lack of resistance or more easily penetrated tissues; a simpler explanation would seem to be that the average actual range of movement of the planont remaining unchanged, the relative smallness of the host would suffice to account for apparently greater penetration. These considerations do not of course affect the amount of infection likely to be found in any particular organ which will naturally be greater in proportion to its proximity to the point of origin, this in the case of contaminative infection being the gut. It is also obvious that those organs immediately surrounding the gut are more likely to be infected than more distant tissues merely owing to their interception of radially moving planonts.

Merogony. The planont, having reached the limit of its range of travel, rounds off and becomes a meront which proceeds to grow and multiply by schizogony in the manner previously described. Judging merely by the numbers present in various tissues, the most suitable environment, and with it the most rapid multiplication, is found in the fat body. In this organ it appears that, once invasion has taken place, the whole mass will rapidly become full of parasites not confined to the particular cell or cells originally invaded but extending throughout all those in the vicinity; it seems indeed that the meront in this tissue is able to pass from one cell into those adjoining, being able to do this possibly on account of the extreme tenuity of the cell walls which may give way either to an enzyme excreted by the parasite or merely to mechanical pressure caused by multiplication and growth of the latter. No direct evidence of this means of spread has been found by the writer nor would it seem probable from the nature of the case that such evidence would be forthcoming. On the other hand, the infection in a fat body is seldom or never sporadic in character but closely compacted, only adjoining cells being affected and in such a manner as to suggest very strongly the gradual spread of infection from one cell to those immediately next to it (Plate X, fig. 4). Examination of sections has not shown any reason for supposing that multiplying meronts are able to break down the outer wall of the fat

body, as this would lead inevitably to their escape in great numbers into the hæmocœlic fluid where, so far as the writer's experience goes, they are but sparsely found in sections of larvæ. It is of interest to note that in some sections of younger larvæ heavy infection of the muscular tissue is found surrounding fat bodies entirely uninfected: in many of these cases nuclear division in the cells of the latter suggests this comparative immunity to be due to resistance arising from active growth. It must be remembered, however, that such apparent resistance to infection on closer inquiry may resolve itself into resistance to multiplication by merogony rather than to invasion by the planont. One alternative to these methods would involve the supposition of a rapid speeding up of the life-cycle of the parasite when situated in the fat body with the production of spores and their germination *in situ*, followed by the production of planonts whose penetrative power would ensure the spread of the disease to the adjoining cells. Another alternative would depend upon the existence of a chemotactic determination of the direction of movement of planonts wandering in the hæmocœlic fluid which would lead to a relatively greater infection of the fat body. The writer has not so far been able to collect evidence in support of either of these alternative hypotheses. Plate III illustrates the absence of spread of meronts into adjoining cells in the case of the epithelium of the gut: here infection is strictly confined to the particular cells in which it was originated by planont penetration. Plate X, fig. 4 shows the very complete infestation of the fat body. Fantham and Porter, in discussing the reciprocal relationships of parasite and host in the case of *Nosema apis*, call attention to the existence of a "halo" or annular ring of altered material surrounding the meront in the epithelial tissues of the bee, and suggest that this represents either, as Stempel thought, solvent action by an enzyme excreted by the meront or a difference in the concentration of the fluid part of the cytoplasm surrounding the latter induced by its absorptive action. These clear annular spaces are not so obvious nor on so large a scale in the writer's paraffin sections but their undoubted occurrence suggests that the action of the meront on the tissues of the host might very well include a solvent one capable of breaking down thin cell walls such as are found in the fat body. A somewhat similar mode of spread appears to take place in the muscles.

Although the parasite very probably excretes solvent enzymes as an accompaniment to, and in furtherance of, the process of merogony, yet it appears certain that no specific toxin is produced at the same time capable of affecting the general vitality of the host. This opinion is based upon observation of the enormous number and general dispersion of the parasites in

individual hosts which may nevertheless continue to live, feed, spin cocoons, come to maturity as moths and perhaps lay a full complement of eggs, even although practically every organ and tissue of the body has been invaded by the parasite.

Nosema bombycis is capable of infecting and apparently multiplying in every tissue of the silkworm, including nerve ganglia (Plate XIII, fig. 1), excepting only the chitinous ones. This power is not only of importance as affecting the general well-being of the host but has a special significance in connection with the hereditary transmission of the disease, inasmuch as it renders probable the entry of the parasite into the generative organs especially those of the female ; this question will be dealt with more particularly in considering hereditary infection.

The most important function of merogony in the life-cycle of the parasite is the multiplication of the latter which takes place during this period ; the rapidity and extent to which such multiplication takes place in the invaded tissues of the silkworm fully makes up for the production of only a single spore (monosporogony) which characterizes alike *Nosema bombycis* and *Nosema apis* as Fantham and Porter have pointed out. It may in fact be argued that this method of multiplication is of advantage to the parasite as merogony takes place under conditions where growth is facilitated by the abundance of nutrient material, whereas sporogony in many cases may be carried out in unfavourable surroundings.

As has been pointed out above, the dissemination of the disease is largely due to accidental distribution of fæces containing spores. It may be of interest to inquire in what manner the latter, formed as a result of merogony in the tissues of the larva, find their way into the unassimilated food thrown out of the alimentary tract. It might be thought that rupture of the epithelial cells of the midgut, as a consequence of their infection and the subsequent multiplication of meronts, would suffice to produce this result, but such rupture does not appear to occur in the silkworms to any marked extent. The irregular growth of the infected cells which may be observed in sections (Plate XI, fig. 1), carrying them sometimes above the line of those in their vicinity, probably tends to produce rupture as a consequence of their interference with the passage of the food in the gut, but the protective action of the peritrophic membrane seems to be adequate to prevent such injury from occurring with sufficient frequency to account for the enormous number of spores which may be found in the lumen of the gut and in the fæces of diseased worms. On the other hand Fantham and Porter have pointed out that in the bee, digestion depends upon shedding of secretory epithelial cells, containing

Fig. 1.

Fig. 2.

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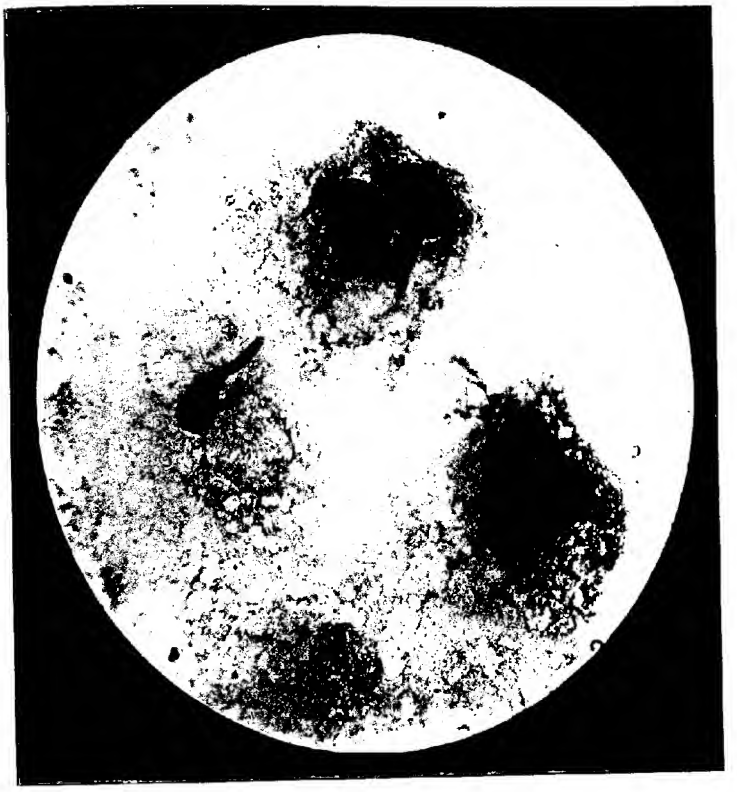


Fig. 3.

Fig. 4.

digestive fluid, into the lumen of the gut, and this process which also occurs in the larva of the silkworm is sufficient to account for the carrying into the gut of such meronts or spores as have been formed in these secretory cells. Plate XI, fig. 2 shows secretory cells full of digestive fluid at the base of the epithelial layer. Plate XIII, fig. 2 shows one such cell following the normal process of outgrowth into the lumen of the gut preparatory to abstriction into the latter. The fortunate circumstance that this cell is full of pebrine spores makes its point of origin and mode of growth particularly clear. In the case of the younger larvæ this mode of outgrowth is sometimes followed by shedding of the complete cell, numbers of which may be found free in the gut (Plate XIII, fig. 3). In the later larval stages apparently only the upper part of the cell is abstricted into the gut where its liquid contents escape either by osmosis or rupture. Such abstricted cells or "cell-buds" are of common occurrence in the lumen of gut sections. Plate XIII, fig. 4 shows an infected cell-bud rupturing in the gut and releasing its contents, amongst them being pebrine spores which would normally pass out with the faeces.

Fantham and Porter (*loc. cit.*) have suggested that this method of excretion might serve as a means of diminishing the severity of the disease in the bee, by throwing out into the gut parasites which would otherwise remain in the tissues of the host; this effect does not seem to follow in the silkworm as the development of infection in the case of young larvæ, in which this ejectory digestive process is most pronounced, is much more rapid (Experiment D) than in later stage caterpillars. Moreover auto-infection would probably be increased in amount in proportion to the extent to which spores were thrown into the gut as a result of this process.

In an alternative process described by Gehuchten for Ptychoptera (*La Cellule*, 1890, VI) the digestive fluid is expelled from the secretory cells by rupture of the latter as a result of increased intra-cellular tension; this also occurs in the silkworm especially in the later stages of larval growth. This digestive excretion takes the form of a thick viscous fluid containing a high percentage of albuminous matter; there is no obvious reason for this transfer of semi-solid material from the secretory cells into the gut in place of the excretion of diffusible ferments by osmosis, but the conjecture might be hazarded that the final release, or perhaps even formation, of digestive ferments from these excreted vesicles depends upon an interaction between the latter and other substances, such as the salivary secretion or possibly enzymes from the food, already present in the gut. This arrangement would tend to simplify the problem of avoidance of autolysis in the secreting cell, and would also explain why pebrine spores which germinate under the influence of the digestive

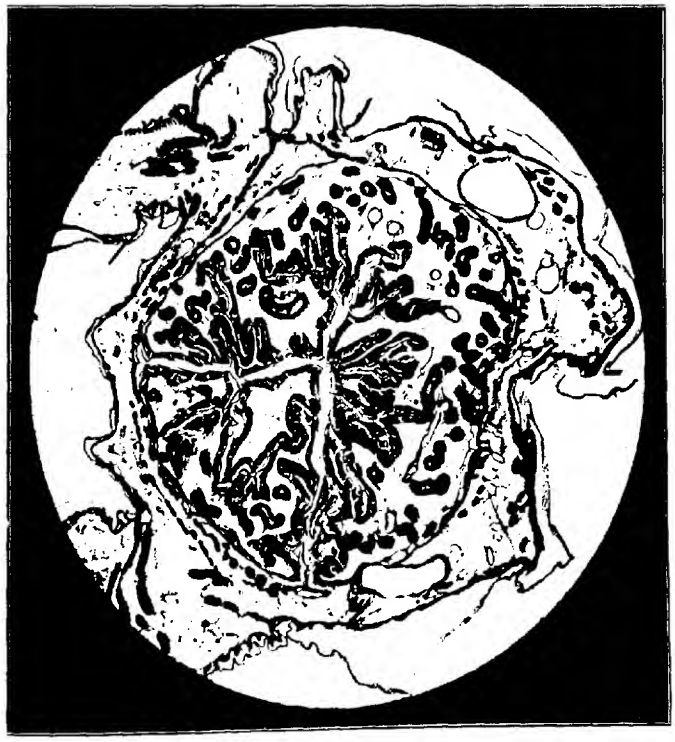
fluid in the gut, do not do so in the epithelial cells producing this apparently physiologically stable secretion.

It must be remembered that in the healthy larva these digestive cells and their secretions when set free in the gut are still separated from the food substances by the presence of the peritrophic membrane. The digestive fluids, or some of them, no doubt pass through the latter by osmosis and so reach and act on the food; it has been pointed out above how this action is interfered with by thickening of the membrane as a result of disease. The spores of the parasite, however, cannot pass through the peritrophic membrane in this manner, and although their occurrence in the fæces might be accounted for by their passage along the annular space between the wall of the gut and the membrane, yet their presence in the lumen of the latter intermingled with the food (Plate XIV, fig. 1) cannot be explained in this way. Dissection of larvæ immediately after moulting shows that the peritrophic membrane is not shed with the other chitinous tissues at the time of ecdysis, nor indeed in view of its mode of formation and endogenous origin would this be expected. Artificial infection experiments, moreover, demonstrate no coincidence between appearance of pebrine spores in the fæces and the time of moulting. Daily examination of fæces shows the constant presence of fragments of the peritrophic membrane therein, and it seems clear that the protrusion of this structure beyond the anus which would follow from its uninterrupted growth is prevented by rupture against the protruberances in the rectum consequent on the strong muscular contractions of the latter (Plate XIV, fig. 2).

Examination of diseased caterpillars, however, shows that in most cases the irregularity in thickness characteristic of the peritrophic membrane even in healthy specimens becomes exaggerated to the extent of complete solution of continuity, in some cases apparently as the result of mechanical rupture. It seems possible that the thickening of the peritrophic membrane caused by the deposition of secretions from the epithelial cells in normal health may be necessary as providing the additional strength required to prevent or repair mechanical rupture by the ingested food. In the diseased condition described above as due to bacterial invasion this natural thickening process is exaggerated until it interferes with the digestion and so with the health of the infected larva. In the case of pebrine, on the other hand, the imperfections and lacunæ observed as associated with this disease may be the result of an opposite process, cessation of the normal strengthening secretions leaving the membrane too attenuated to withstand the rupturing tendencies of any hard fragments of the food. Owing to the folding of the membrane it is very difficult to follow its outline and determine its continuity or otherwise, even in transverse

Fig. 1.

Fig. 2



sections, and in most longitudinal ones this is impossible even when strictly median sections are selected. Dissection and sectioning, however, demonstrate the fact that the peritrophic membrane in pebrinized larvæ is imperfect and easily ruptured, and it seems probable that rupture commonly occurs in such cases through the mechanical strains set up by the combined action of hard portions of the food, such as midribs of the leaf, and muscular contractions of the gut incidental to peristalsis. Through such ruptures or lacunæ the accumulated masses of spores will pass from the annular space between the epithelial cells and the peritrophic membrane into the lumen of the gut and from thence will be carried with the undigested residue of the food into the proctodœum and finally thrown out with the fæces. The writer does not feel satisfied that this explanation is completely sufficient to account for the appearance of great numbers of spores in the fæces within a few days of artificial infection with ingested spores. There is no evidence in support of the theory of merogony followed by sporogony in the lumen of the gut itself which might account for such multiplication, nor for the alternative one which supposes that such merogony might take place in the œsophagus, the resulting spores being then carried with the food through the œsophageal valve into the lumen of the mesenteron. There can be no doubt that infection through ingested spores takes place first in the midgut, and the spores appearing later in the fæces must be the result of merogonal multiplication in the epithelial cells of this portion of the alimentary tract. One other explanation suggests itself as possible; owing to the close approximation between the peritrophic membrane and the epithelial cells of the gut wall when food is present in the stomach it is conceivable that the protrusion of the secretory cells above described and figured, might lead to rupture of the membrane and the passage of these cell-buds, with any contained spores, through the latter direct into the lumen of the gut. Unfortunately the writer has not so far been able to obtain conclusive evidence of such a happening, although the presence of such cell-buds in the gut apparently within the circumference of the peritrophic membrane would lend support to such a conclusion.

Experiments to determine the period of time between artificial infection by ingestion of spores and appearance of the latter in the fæces show that this is of shorter duration than in Europe (Pasteur, *loc. cit.*) and suggest a more rapid passage of the parasite through the various stages of its life-cycle in this country, probably as a consequence of the higher average temperatures. The bearing of this factor upon methods of combating the spread of infection requires further investigation together with that of the resistance of the spore to outside influences tending to destroy its

infective capacity. A beginning of this investigation has been made and has included some experiments to determine the effect of desiccation, of time, sunlight and antiseptics (Experiments K, M, N) upon the viability of the spore. Other experiments have aimed at getting some quantitative measure of infective capacity (Experiment R) mainly with the idea of developing a method for future use in connection with the inquiry into modes of eliminating contaminative infection, but also with a realization of the value of importing quantitative methods into biological research whenever this is possible.

Hereditary Infection results either from infection of the ovum by penetration of the parasite (true hereditary infection) or from contamination of the egg by contact with infected parts (ovary or ovipositor) of the female moth. Infection of the ovum may involve (1) actual invasion of the tissues of the embryo, (2) mechanical inclusion of parasites in the embryonic gut cavity by invagination or otherwise during development of embryo, (3) contamination of young larva, just before emergence by ingestion of the residual yolk mass containing spores. Probably infection of the ovum itself can only be effected by the actual motile form (planont) of the parasite and this can only occur before formation of the chorion, *i.e.*, in the pupal ovary. The possible spread of infection from one cell to another, not by planonts but by meronts, as a consequence of enzyme secretion by the latter, seems to take place in the fat body as suggested above and may happen elsewhere, but is not likely to explain ovarian infection nor movement of the parasite in the egg from yolk to embryo ; this would probably require not only a concentration of the enzyme only obtainable as the result of close contact between the parasite and the cell wall of the tissue to be invaded, but also pressure upon the latter resulting from crowded growth of meronts in the originally invaded cell ; this condition would not obtain in the egg.

There is no conclusive evidence of infection through the micropyle as the result of fecundation whereas there are numerous cases of infection in the pupal ovary. Ovarian infection has been observed in the pupal ovary both as recently entered planonts though small in numbers, numerous meronts resulting from division of the former, and as spores.

Planonts, so far as can be judged from observation, probably do not retain their motile character on entering suitable tissue but rapidly become meronts and undergo schizogony, nor do they remain in the gut of the larva for any time after emergence of the amœbula from the spore but at once make use of their motility to penetrate the gut wall to distances varying with their original store of motile vitality, and with the thickness of tissue and resistance to penetration. It does not appear probable that all the meronts found in

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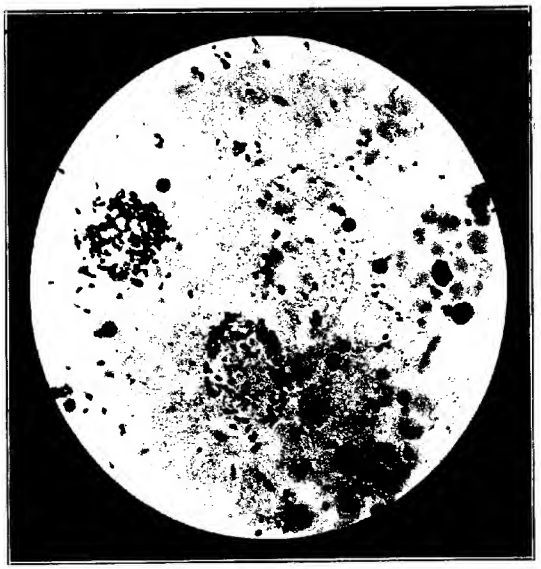

Fig. 2.

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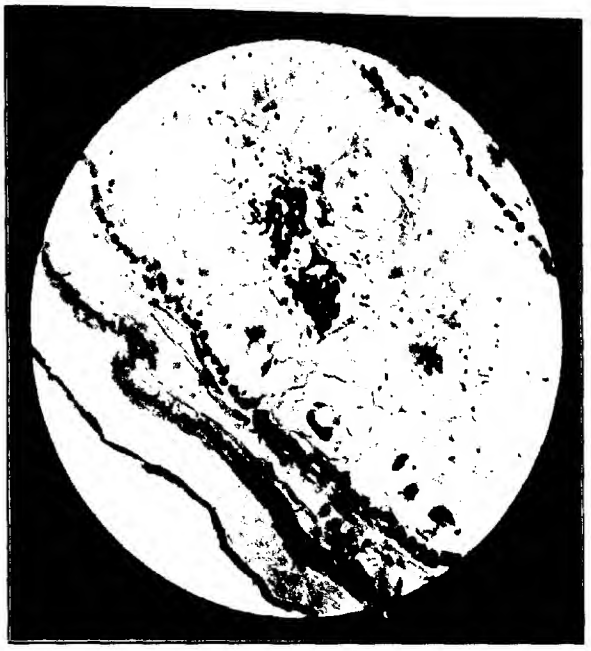

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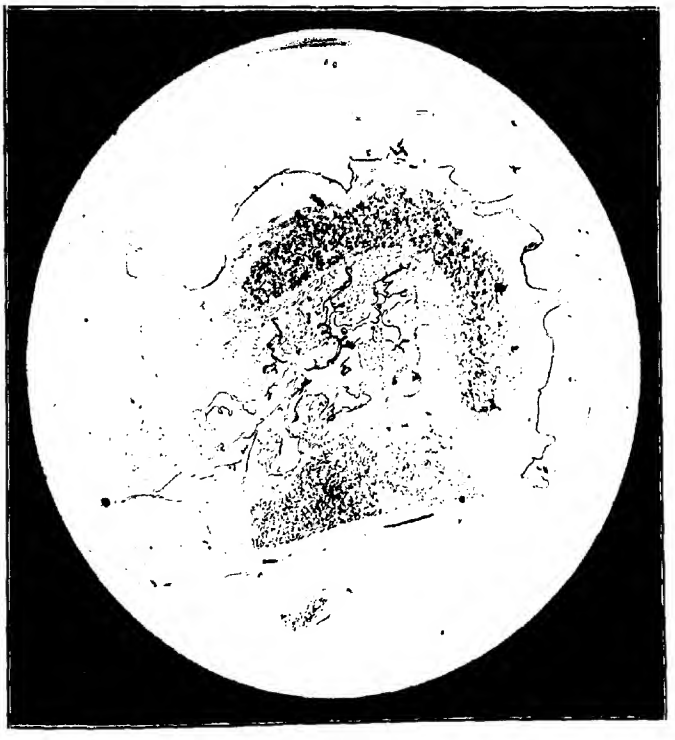

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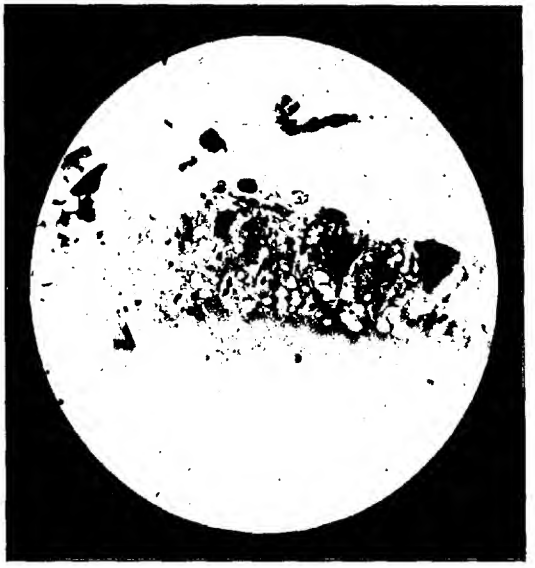

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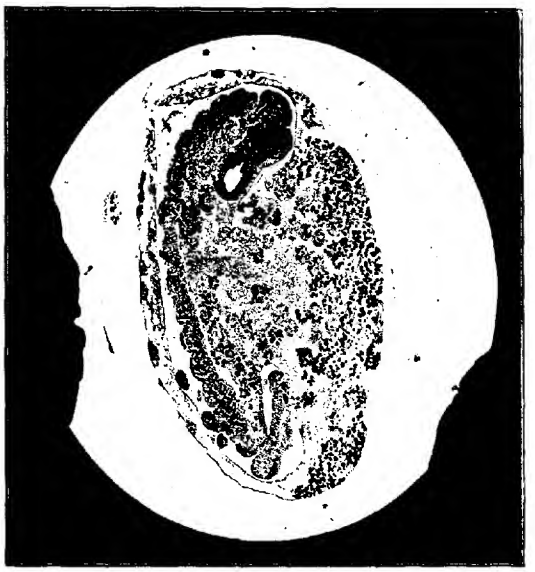

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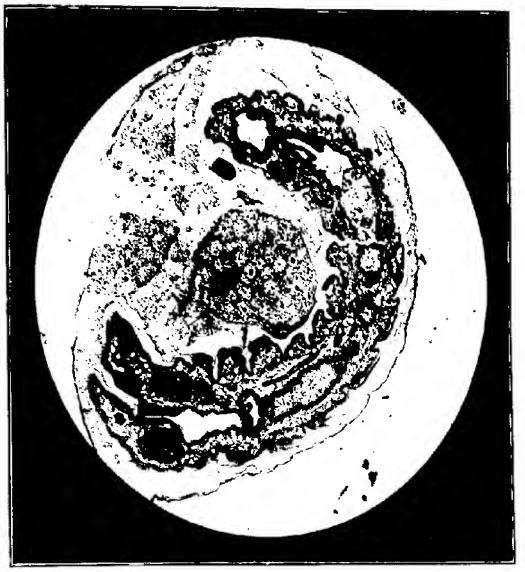


Fig. 3

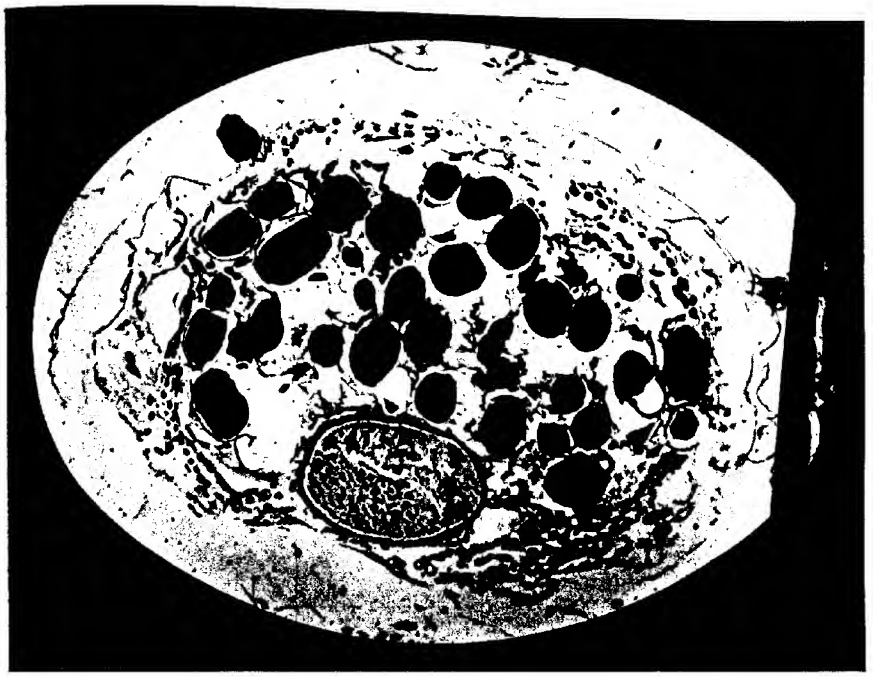
Fig. 4

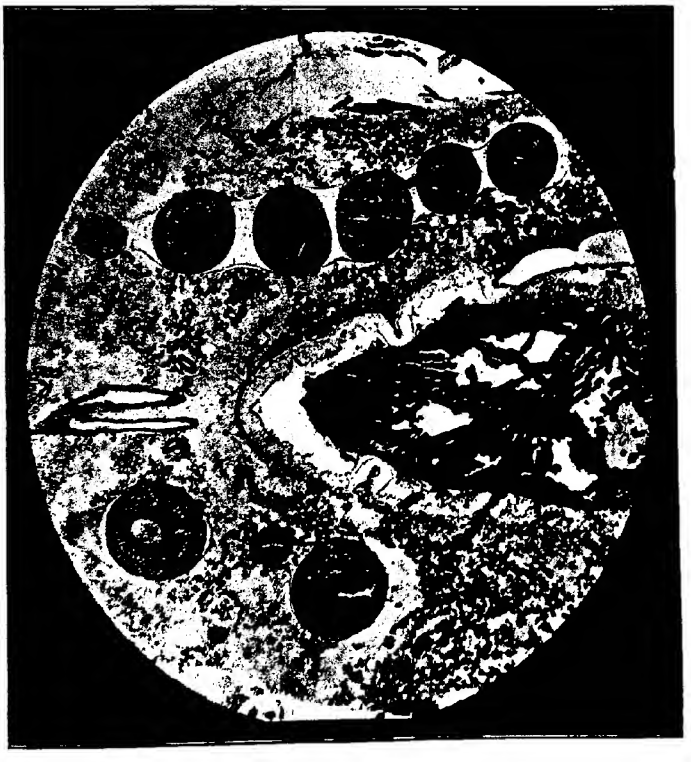
the pupal ovum arrived there as the immediate result of the germination of spores in the larval gut, as this would necessitate the assumption either that the originating planonts had existed as such in other parts of the larva or pupa for several days before penetrating the ovum itself or that they remained in the latter situation for this length of time without undergoing the usual merogony with its accompanying degree of multiplication. These are the alternatives demanded by the currently accepted theory of the cause of germination of the spore, *i.e.*, that this only occurs as the result of the stimulating action of the digestive fluids of the larval alimentary canal. On the other hand it is easy to find numerous planonts in the gut cavity or yellow body of the pupa after as long a period as ten days' pupation (in the cold season in India), *i.e.*, in the absence of digestive fluids as such; these planonts no doubt give rise to the ovarian infection above referred to as well as to that of other inguinal organs in the process of formation. It appears probable that the new crop of planonts owes its origin to the germination of spores in the pupa induced by the stimulating action of the various enzymic secretions characteristic of the extensive amœbocytosis accompanying the metamorphosis, and this supposition would account for the occurrence of obviously recent infection in such situations as the interior of the ovarian follicle and of the ovum itself. Sections of infected eggs show the presence of parasites, mostly as dividing meronts, during the earlier stages of the development of the embryo (Plate XV, fig. 1), whilst spores are found to preponderate later (Plate XV, fig. 2). Now although numerous parasites may be found both in the egg and in the just hatched larva, in the majority of instances these are mainly confined in the former either to the yolk mass or to the alimentary tract of the embryo, and in the latter to the lumen of the gut (Plate XV, figs. 3 and 4). In some instances however we find actual invasion of the tissues of the embryo itself and it is difficult to avoid the conclusion that such invasion implies the presence of planonts in the ovum itself, and consequently that the germination of spores may go on in the yolk mass or elsewhere. It is of course merely a matter of conjecture whether such germination is effected by enzymes associated with the ovum as a whole or actually secreted by the developing embryo, but the actual presence of unmistakable spores in the cells of the wall of the gut of the embryo only five days after oviposition as shown in Plate XVI, fig. 1, suggests the former alternative as necessary in this instance at any rate.

Numerous cases are found in just hatched larvæ of infection of other tissues besides those of the gut, these also supporting the supposition that actively motile planont forms of the parasite are present in the egg at a late stage of development.

The invagination characteristic of the formation of the embryo in the egg of the silkworm accounts for the merely mechanical inclusion of numerous parasites present in the cytoplasm or yolk mass and the consequent relatively large number found in the gut of infected larvæ recently hatched, but the number of parasites in this situation is certainly added to in many cases by the habit of the larva of swallowing the remains of the egg contents just before emergence (*Tut. Br. Lepidopt.*, Vol. I, 1899) (Plate XVI, fig. 4). Larvæ starting life under such conditions will seldom go through more than two or three moults and will never spin cocoons, much less carry on to the next generation as egg-laying moths. This early doom is *a fortiori* inevitable for those in which the tissues have been invaded whilst still in the embryonic state. A characteristic feature of infected eggs is the marked preponderance of meronts over spores in the majority of instances examined. This would follow naturally supposing infection of the ovum to occur mostly in late stages of its development, as appears to be probable. The importance of this point so far as Indian sericulture is concerned lies in its bearing upon the use of microscopic examination of the egg as a means of detecting disease. As has been pointed out above in the multivoltine races so largely used in India, the egg hatches out in less than ten days after oviposition, thus cutting down the time for examination of the eggs to a period which is extremely short when compared with that possible in European countries, where this method of detection of pebrine is sometimes used as a subsidiary additional one. As the unstained meront is practically indistinguishable and in such a rich nutrient medium as the egg sporogony is probably considerably delayed, many highly infected eggs might escape detection if examined within a few days after laying, as they must be in this country, and for this reason alone egg examination by the ordinary microscopic methods can never become a practical method in Indian sericulture; it appears doubtful whether, even in Europe, it can afford any really reliable indication of the presence or absence of pebrine.

The infection of the ovary and subsequently of the egg itself is naturally a matter of chance, but this chance will depend largely upon the extent of infection of the larva, *i.e.*, upon the actual number of parasites present in the latter when it arrives at the pupal stage. This again will be determined by various factors such as the number of spores ingested with the food, the earliness of the stage at which this takes place, and the extent to which natural resistance comes into play. The egg tubes in the later stages of pupation occupy a very considerable proportion of the body cavity and are so disposed round the alimentary canal as to form a likely mark for planonts emerging





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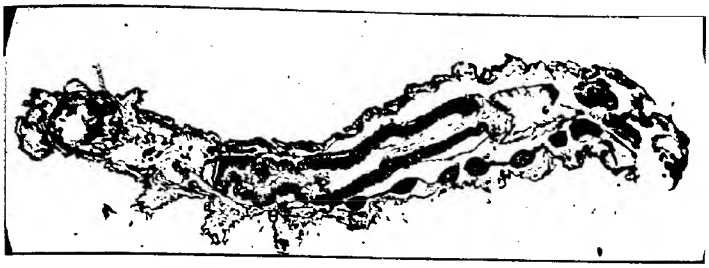

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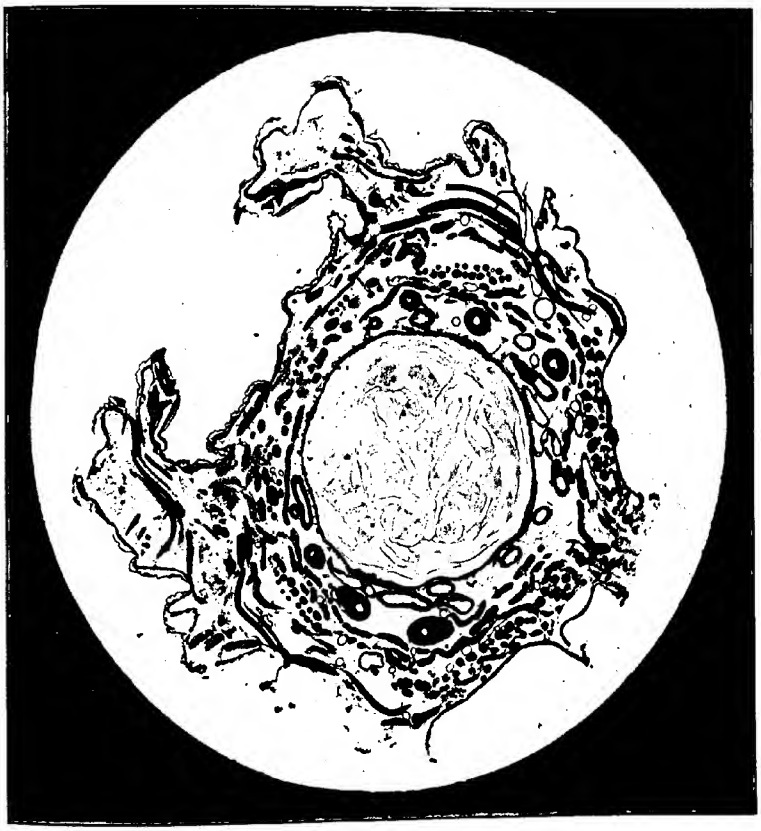
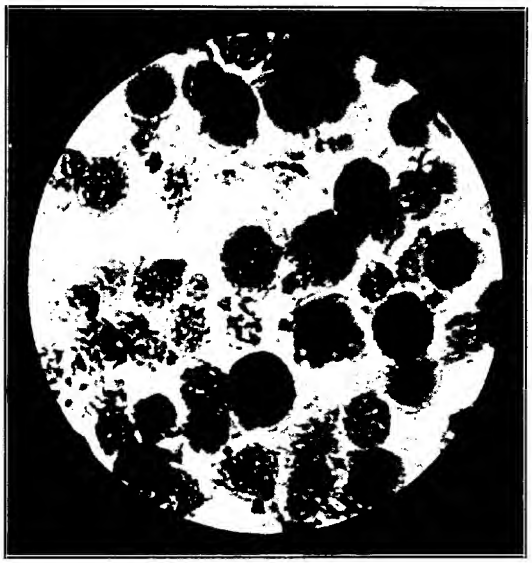


Fig. 2.

Photo. engraved & printed at the Offices of the Survey of India, Calcutta, 1926



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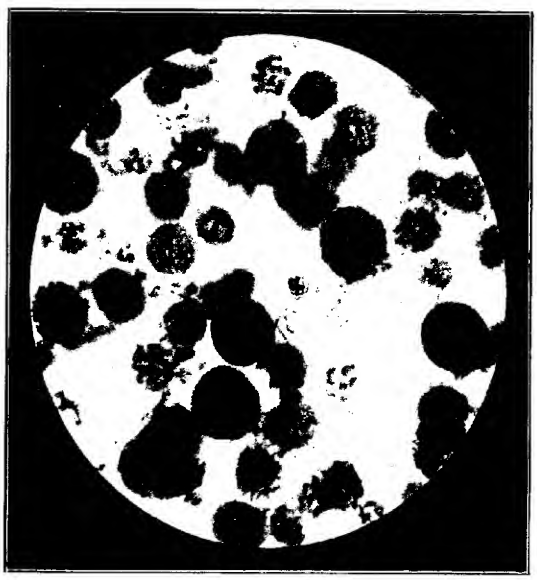

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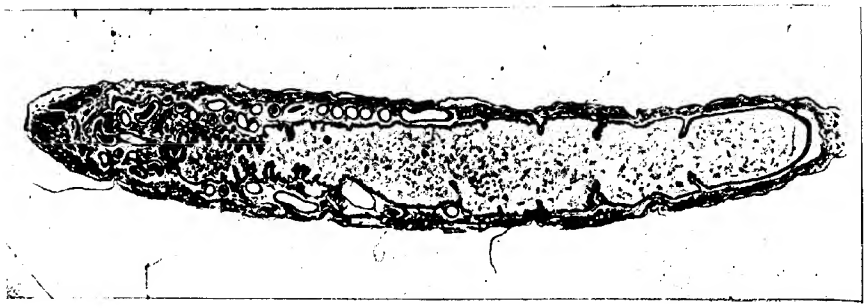

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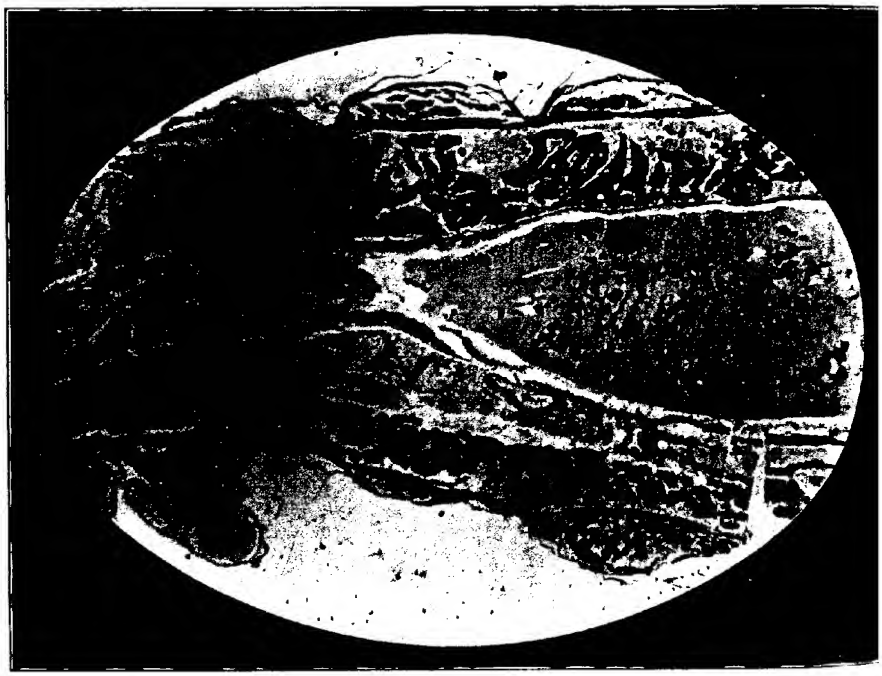


Fig. 2.

Fig. 3.

through the walls of the latter (Plate XVII), just as is the silk gland in the larval stages of development (Plate XVIII, fig. 2). This fact forms an additional reason for supposing that examination of the gut of the moth, derived as this organ is directly from that of the pupa, will afford good evidence of probable infection of the eggs should parasites be found by such examination.

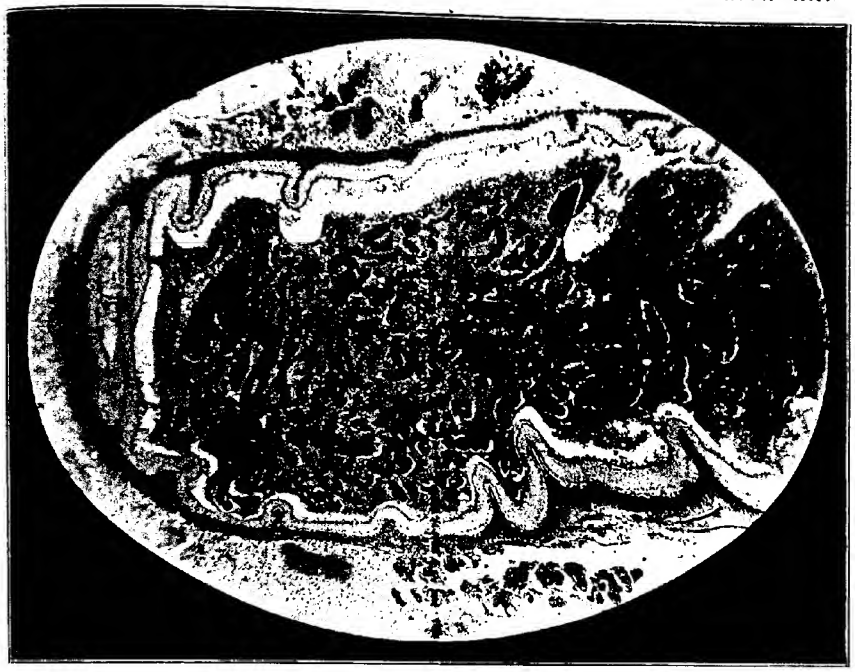
The Metamorphosis. The derivation of the gut of the moth from that of the larva in the Lepidoptera, referred to above, is of course a fact well known to entomological science. In the case of the silkworm it is illustrated by Plates XX to XXIV, which are of particular interest in connection with this enquiry as showing the rapid disintegration of the original superficial epithelial lining of the larval mesenteron and its replacement early in the metamorphosis by a new set of cells. The old cells are separated from the underlying new ones by a division apparently very similar to that which takes place when the middle lamella of vascular plant tissue is destroyed by the secretion of pectase by an invading bacterial parasite. The old cells come away as a more or less complete layer (Plate XXIII, fig. 2) sometimes as early as within thirty-six hours from commencement of pupation in the summer or forty-eight in the winter. Within twelve hours from this abscission, however, disruption appears to set in and in another forty-eight hours the layer of old cells has broken down into fragments in which the structure of the original cells can hardly be distinguished. At the end of the period of pupation, which varies in India from 18 days in the cold weather to 8 days in the hot weather and monsoon, the gut cavity of the pupa contains only a mass of structureless material (the yellow body) (Plate XXII, fig. 2) which apparently loses some of its moisture, but persists with its original content of pebrine spores directly derived from the larval epithelium (Plate XXV, fig. 2), as the dark brown mass found in the gut of the moth. This brown mass gives to the moth's stomach its characteristic colour, easily seen through the thin walls of epithelium surrounding it, and thus rendering easy its identification as the part for examination by the writer's method (*loc. cit.*). It is to be noted that no voiding of the contents of the stomach or intestines takes place during or after pupation, so that the broken-down remains of the original epithelium with any content of spores originally present will be found in the stomach of the moth. It may be of interest to mention that in the multivoltine silkworm the new epithelial lining tissue of the imaginal gut does not seem to originate from separate imaginal buds situated at intervals around the circumference of the intestine but appears to be formed as a continuous outgrowth or proliferation of the basal cells of the original secretory layer; this method

of formation will help to account for the very heavy infection sometimes found in the epithelial cells of the moth's gut (Plate XXIV, fig. 2.), as it is not necessary to assume that most of the parasites found in this situation only arrived there at a comparatively late stage of the metamorphosis, as it would be if the theory of the development of these cells from isolated imaginal buds were adopted.

During the metamorphosis the development of the ovary is carried to a point at which the formation of the ova arrives at an advanced stage; it has been shown that the situation of the ovarian tubes surrounding the gut must render the former highly liable to invasion by planonts originating in and leaving the latter, and it has been suggested above that the active amœbocytosis proceeding in the pupa may very well be a direct cause of germination of any spores present at this stage. This suggestion is supported by examination of material derived from the pupal gut and by the demonstrably active infective nature of such material, which further negatives the idea that the ferments characteristic of amœbocytosis have any serious destructive effect upon the parasite.

The presence of recently entered planonts in ova in the pupal ovary (Plate XIX, fig. 1) containing no other form of the parasite appears to be sufficient proof of infection at this stage, and their probable origin in the gut would emphasize the importance of this organ as a focus of the disease. It has already been argued that invasion of the ovum itself, as distinct from the epithelial cells of the ovarian follicle, can only result from the presence in the neighbourhood of the ovary or in the latter itself of active planont forms of the parasite after the ovum has been formed and before secretion of the chorion. An alternative but unlikely method above referred to would depend upon invasion of the ovum as a result of merogony in the epithelial cells of the ovarian follicle accompanied by breaking down of the intervening cell walls in a manner similar to that apparently taking place in the fat body and above discussed. The much more substantial tissues involved even in the pupal ovary (Plate XIX, fig. 3) seem to render this supposition an improbable one. No doubt in cases of invasion of the egg tubes such as that illustrated in Plate XXVI, fig. 2, showing heavy infection and hypertrophy of the lining epithelium in the egg tube of a diseased moth serious contamination of the eggs during oviposition would occur, but this would be entirely superficial.

It has been found by experiment at Pusa that infection of the eggs of a moth could sometimes be obtained by artificial infection of the last full meal of the larva, *i.e.*, some twelve hours before spinning (Expt. O, No. 4). In such cases the actual amount of infection present in the



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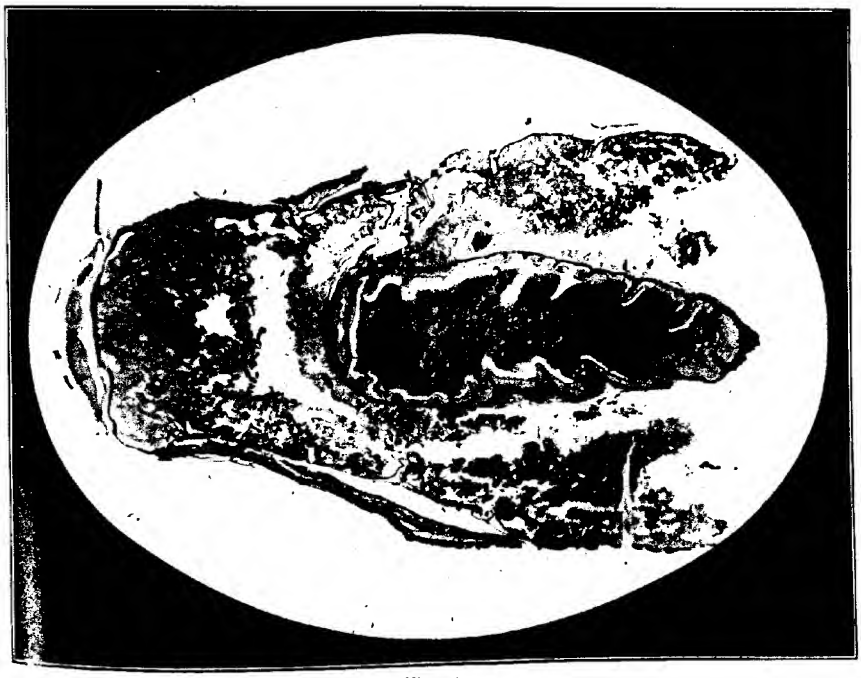

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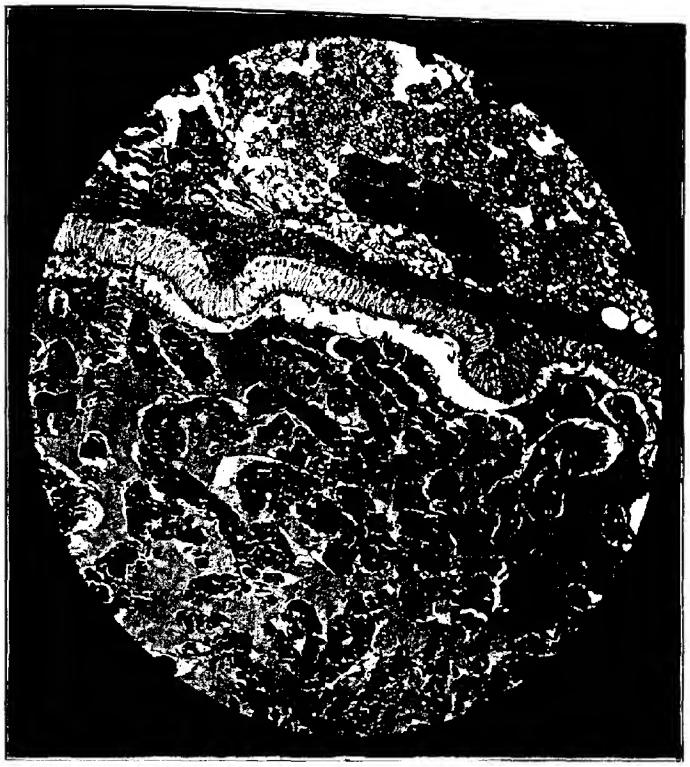

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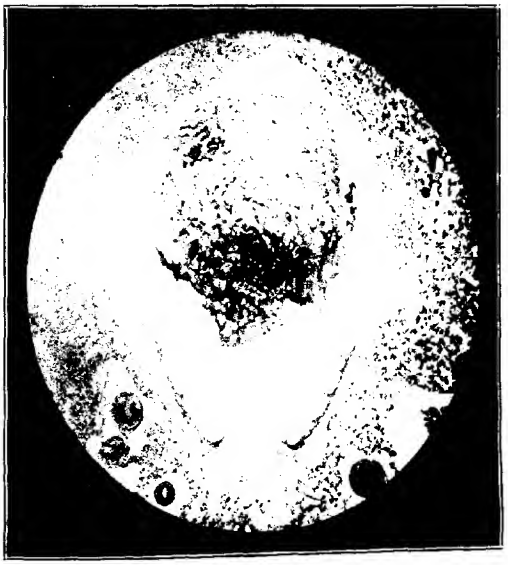

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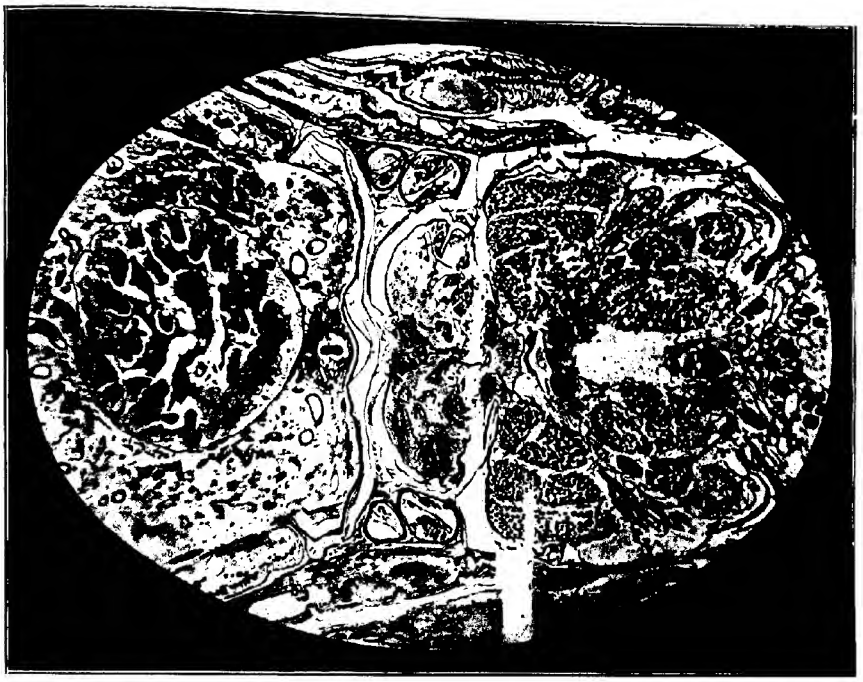

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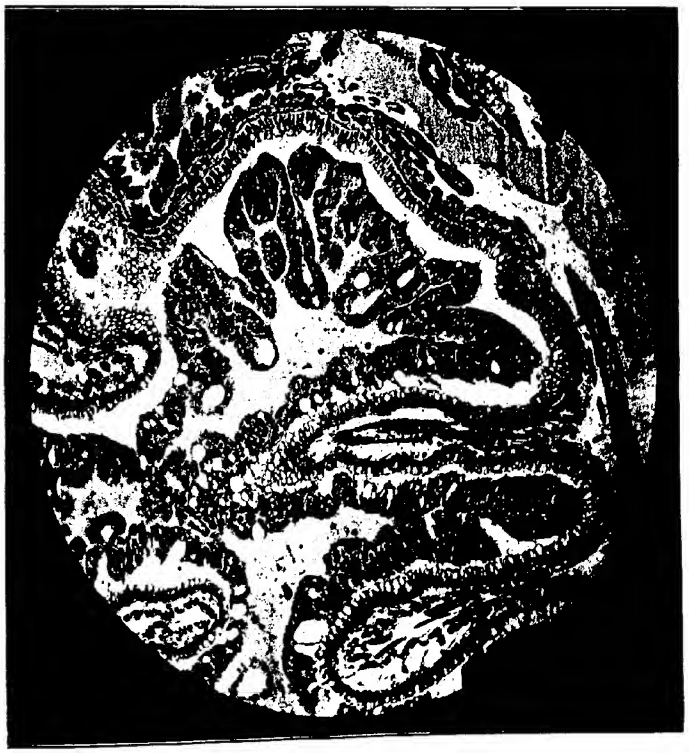

Fig. 1.

Fig. 2.

PLATE XXV

Fig. 1.

Fig. 2.

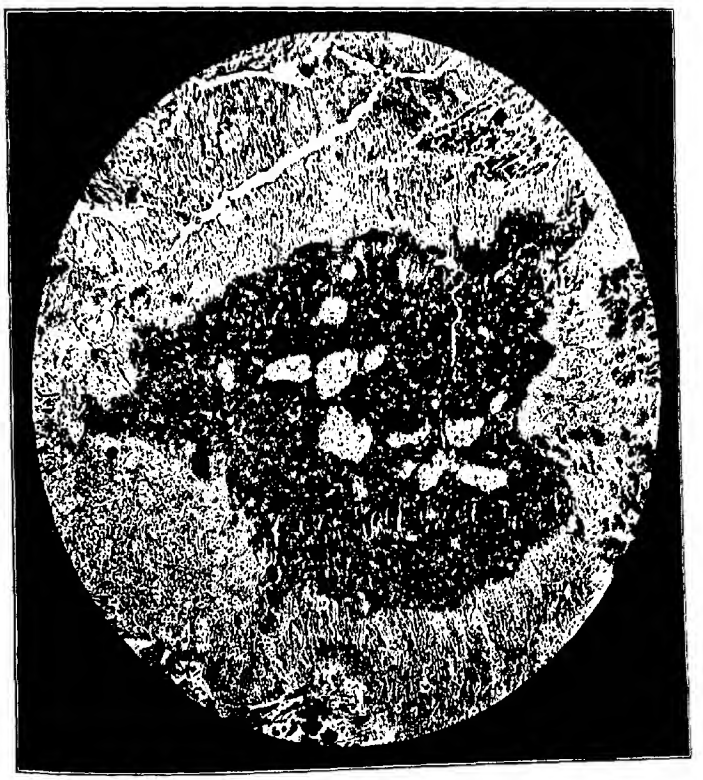

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

moth will necessarily be small and so will be the chances of detecting it by the ordinary method. Plate XXVI, fig. 3 shows the presence of infection in the gut tissue of the pupa six days after inoculation. In Plate XXVI, fig. 4, infection is seen in the gut of the moth; these specimens were taken for sectioning from amongst 30 individuals in the experiment referred to above. No spores could be found in any other part of the moth except a few in the Malpighian tubes and the smallness of the whole amount present would render detection by the total trituration method a matter of considerable hazard. Here then we have a case in which hereditary transmission occurs in spite of the lateness of the time of infection and of the consequent paucity of parasites present which would, in default of actual evidence to the contrary, seem to negative such a possibility. Although a smaller percentage of infection, both of moths and their progeny, may result from such late entry of the disease yet this infection and hereditary transmission can and does occur under these conditions, and unless a method of examination is made use of capable of detecting it in the moth the seed will be passed as disease-free and will go out to infect the otherwise healthy broods of the rearer unfortunate enough to have it foisted on him.

It was stated earlier in this paper that reasons existed for thinking that the gut of the moth might be considered as that portion of its body in which infection would most easily be detected by the ordinary microscopic method of diagnosis as adopted in the grainage. This argument is based upon the following considerations drawn from the observations upon the mechanism of infection above recorded and described:—

(1) Meronts are not recognizable under the ordinary microscopic diagnosis; only the spore form of the parasite can be detected in this manner.

(2) Since the processes of merogony and sporogony require the lapse of time to bring them to completion, spores are the more likely to be found in tissues in proportion to the period of time since the invasion of the latter.

(3) Spores are therefore most likely to be found in those tissues earliest infected, especially as in these situations merogony will soonest be brought to a conclusion by failure of the food supply and will be followed by sporogony.

(4) The tissue earliest infected is the epithelium of the midgut of the larva; infection can be demonstrated as occurring first in this situation, by the presence of meronts, and later of spores both in fresh preparations and in microtome sections.

(5) Study of the metamorphosis demonstrates clearly the identity of these epithelial cells of the larva with the gut contents of the moth.

(6) Owing to the relatively mature condition of the spores in the epithelial cells of the gut auto-infection is more common in the neighbouring tissues than in other parts of the host; in the moth this process appears to result in the infection of the new epithelium of the gut so that this part of the imago becomes a centre or focus of concentration of the parasite, not only by reason of the persistence of original spores in the lumen, but of the formation of a fresh supply as the result of auto-infection.

Reasons have also been given for thinking that this collection of spores in the gut of the moth may be added to by the sporogony of parasites originally present in the basal epithelial cells of the larval mesenteron.

Reference has already been made (p. 183) to the uneven distribution of the parasite in infected moths and the consequent possibility of overlooking disease in examining lightly infected individuals. Numerous instances have come within the writer's experience of infection occurring only in the gut of the moth and not apparent in outlying tissues. In the writer's opinion such localization of the disease, although implying very light and probably late infection, does not remove even the probability of infection of some at least of the eggs, owing to the close proximity of the pupal ovary to the gut as a centre of infection. If this opinion, based as it is upon the observations recorded in this paper, constitutes a sound deduction from the facts reviewed, there appears to be no escape from the conclusion that the elimination of hereditary infection as a source of pebrine in the multivoltine silkworm in India cannot be effected by the Pasteur method as practised in Europe.

In dealing with the elimination of pebrine it is very necessary to appreciate the fact that the spread and persistence of this disease are contributed to both by hereditary transmission and contaminative infection. It must be remembered, however, that although one diseased individual as an egg-laying moth may be responsible for the production of 200 diseased larvæ, yet the main increase in number of affected worms will be due, especially in India, to the contamination by the infected fæces of the hereditarily diseased individuals of the food of all the otherwise healthy caterpillars in their vicinity. Were it possible, or rather practicable, to eliminate contaminative infection completely the disease would almost certainly disappear, as hereditary infection cannot continue through three consecutive generations owing to the rare survival to the egg-producing stage of larvæ infected *ab ovo*. Elimination of hereditary infection, however, although it would, if complete, be an immense step in the direction of elimination of the disease itself, would not necessarily have this result. The parasite if present in the rearing houses at the outset could be carried on, in India, from one generation of silkworms to the next and from

one season to the following one, producing disease in each, merely by the contaminative processes described above; the incidence of the disease would no doubt be greatly reduced and the consequent mortality lessened owing to the absence of ovarian transmission, but the disease would always be there so long as no effective measures were taken to reduce or abolish purely contaminative infection. The retention of virulence by the spore form of the parasite over as long a period as six months in the plains of India, as shown by the results described in Experiment K–15, makes clear the most serious difficulty met with in dealing with the elimination of pebrine in this country. Although it is quite probable that this maximum period observed is an exceptional one, and also that a high percentage of the spores present in the dust of a rearing house lose their effectiveness as producers of disease after a shorter period of time, yet there can be no doubt that in the absence of efficient means of sterilization, the disease can be carried over in India from the end of one season to the beginning of the next by the persistence of infection in this form. We have here again a difference between European and Indian conditions, unfavourable to the industry in the latter country, and due to the prolonged period of rearing consequent on the numerous broods of the multivoltine races. In Bengal rearing may begin in March and continue until November, thus leaving a comparatively short period for infection to die out. This subject requires careful investigation in order to arrive at a just estimate of the relative importance of hereditary and contaminative infection and of the possibility of dealing effectively with the latter as well as with the former. It does not appear probable, however, that the persistence of pebrine in Europe after fifty years of examination of moths by the Pasteur method is due entirely to contaminative infection, and this is certainly not the case in India. Contamination is due to the resistant nature of the spore form of the parasite and the only apparent method of avoiding it would seem to depend upon antiseptic measures resulting in the destruction of all spores present in the rearing houses. It will be readily understood by any one conversant with Indian conditions how nearly any such method of dealing with the problem approaches the impracticable; the spores themselves in incalculable numbers are distributed over every exposed surface both of floor, walls, ceiling, and rearing trays. In Europe where stone or brick with perhaps wooden floors and ceilings present cleanable surfaces, contamination may be kept within bounds by periodic disinfection, which is also rendered easier by the fact of only one brood being raised in the year; in India, however, the numerous broods of the multivoltine races commonly in use not only enormously enhance the actual amount of infective material produced, but reduce the opportunities for

getting rid of it by disinfection. The silk industry in India, moreover, depends upon the success as silkworm rearers of such individuals of the cultivating class as find time and inclination to practise an art the knowledge of which has been handed down merely as a cottage industry subsidiary to agricultural practice. Men of this class depend largely upon their womenfolk and smaller children to conduct the various operations connected with the rearing of the worms, and these latter are generally carried out in the more or less vacant spaces to be found in houses built as a general rule entirely of mud, bamboo, thatch grass, and cowdung; the floor and walls being of earth provide great quantities of dust and harbour innumerable parasites, which are carried by currents of air so as to infect thoroughly everything in the dwelling, including the clothing of the rearers, who in this way spread the disease from their own houses to others which they may visit. Very little of any practical value can be done to remove contaminative infection from the rearing houses of this large class of producers of raw silk*; the only help that can be given to eliminate pebrine is to provide them with disease-free seed and information as to the value of ventilation, of proper spacing and diet in enabling the worms to arrive at the spinning stage in spite of the infected surroundings which seem unavoidable in houses of this class. It is obvious that seed cocoons raised in such surroundings must inevitably produce a high percentage of diseased moths, and that the best chance which exists for the improvement or even the survival of the silk industry in India rests with the provision of an unfailing and reliable supply of disease-free seed. Even in the case of the univoltine races this has been abundantly demonstrated by experience in Kashmir, where the success of the industry has been practically bound up with the yearly importation of fresh seed from Europe. Much more is it likely to be true for the multivoltine races of the plains with the cumulative infective effect of the successive broods. No intermediate host appears to be necessary for the completion of the life-cycle of *Nosema bombycis* as we know it at present, and as a consequence of this fact and of the still more important one that the cycle itself can be completed within a few days' time, as shown in the experiments on artificial

* The writer has suggested the use of paper linings for ceiling and walls in the rearing houses, with the idea of burning these at frequent intervals and so keeping down the accumulation of infective material, but owing to the present high war price of paper it has not been possible to experiment with this method on any large scale. The principal difficulty remains in the nature of the floor and the impracticability of preventing dust by watering this. Something may probably be done by the use of calcium chloride to overcome this trouble, the hygroscopic nature of this salt making it eminently suitable for ensuring a constantly moist condition in earth floors watered with a solution of it, at any rate for several months of the year.

infection above cited, the introduction into a rearing house of a single diseased individual will inevitably lead, in the absence of adequate antiseptic measures, to infection which may, and probably will, persist as a source of disease to a constantly increasing percentage of all subsequent broods in the affected house.

Some idea of the importance of contaminative infection in India may be gathered from a general description of the methods of rearing the multivoltine races as practised in Bengal. In a large number of districts the rearers prejudice their chances of starting with disease-free seed from the outset by refusing to take this in the form of layings of eggs direct from the grainage, and insisting upon being supplied with seed cocoons. Their principal reasons for this are that they wish to be assured of the quality of the silk and at the same time to get some idea of the healthiness of the stock from the character of the cocoon. The result, however, is that although the seed from which such cocoons were raised may have been disease-free at the time of oviposition, there is no certainty that infection is absent from the cocoons, derived from contamination occurring at some period of the intervening larval stage. The rearer in Bengal raises five main broods in the course of the year: (1) March, (2) April-May, (3) June–July, (4) August–September, (5) October-November. In addition to these he may also rear subsidiary intermediate broods for seed and in some cases a winter rearing is also carried out in December January-February. Now if the rearer carried out complete disinfection of his rearing house between the broods, and obtained a fresh supply of disease-free seed from the Government grainage for each one, the cumulative effect of the contaminative infection above referred to as being of such importance would not come into operation. This, however, is far from being the case in Bengal. The rearer, beginning as shown above with possibly infected seed cocoons, proceeds to rear a more or less continuous series of broods throughout the year, each one adding enormously to the possibly small amount of infective pebrine spores originally present, and each successive one inevitably containing a higher percentage of diseased individuals owing to its derivation from a predecessor reared in the midst of contaminative infective material. It has been shown that one feed of infected leaf even as late as the last day of larval life is capable of initiating hereditary infection, and although this is probably an exceptional case, still there can be no doubt as to the extreme rapidity with which the combination of hereditary and contaminative infection can produce and spread the disease under the conditions obtaining in the rearers' houses in Bengal, and indeed in any part of India where the multivoltine race is made use of without strict preventive measures.

It will be obvious from consideration of the above conditions of rearing that the importance of providing the Indian rearer with truly disease-free seed can hardly be exaggerated. In the writer's opinion the present want of confidence in nursery seed exhibited by the Bengal rearer is directly to be traced to the faulty method of examination which has for so long been made use of in selecting it. Had a really effective and critical method been adopted it is probable that the rearer's experience of its reliability would have taught him not only to make more general use of it than is found at present, but would quite probably have led to his preferring it to his own for carrying on from one brood to the next in mid season. It is also probable that the experience of such rearers as began in newly built houses with *completely* disease-free seed would have had so great an educative effect in teaching not only confidence in the Government grainages, but also some glimmerings of the value of cleanliness, that great advances might have been made in Bengal, as they have been made in other parts of the Orient. No greater mistake in policy in dealing with the conservative minded Oriental peoples can be made than the introduction for their use and ostensible benefit of a Western method before adequate investigation of its appropriateness to Eastern conditions. The Pasteur method was hastily introduced into Bengal without consideration of the bearing of the wide discrepancies between European and Indian conditions or apparently any knowledge of the scientific facts connected therewith. After nearly twenty years of its use we find pebrine rampant not only in the districts but in the nurseries themselves, the confidence of the rearers in grainage seed practically non-existent, and the silk industry as a whole at a low ebb, as Professor Lefroy reports, mainly on account of the ravages of this disease. How far this condition of affairs may be due to improper use of the European method and how far to its intrinsic unsuitability to Indian conditions it is difficult to say, but the fact remains that without some drastic alteration in the method of providing disease-free seed the silk industry in India can only go from its present decadent condition to one of complete decay. The success of the industry in India like that of the majority of others in this country depends mainly upon the cheap and almost incidental production of the raw material; the cheapness again depends largely upon the availability of low paid labour, that of otherwise unemployed women and children generally associated with cottage industries such as this may be considered. The cheapness of the raw material also depends upon the possibility of growing mulberry on land which cannot be more profitably occupied by other crops. It is this condition which is easily interfered with by the failure to provide disease-free seed, the use of which may frequently lead, when the worms,

owing to development of pebrine, live through all their larval stages only to fail to spin, to loss not only of the labour of weeks but of the value of the mulberry leaf consumed. It cannot be a matter for surprise that as a result of such disappointment the rearer should turn from sericulture to more profitable or at least less uncertain agricultural occupations, and that the land under mulberry cultivation in Bengal has steadily lessened in area. Much work remains to be done in this country to determine the possibilities of coping with infection both hereditary and contaminative; no doubt such work will include observations on the increase of natural resistance to infection, either by hybridization, selection, hill rearing ("amelioration") or improved hygiene; very encouraging results on these lines have already been obtained by the writer, although the experiments have not been carried far enough at present for conclusive report. One important aspect of future work must be the investigation of methods, either natural or artificial, of disinfection of grainages and, so far as may be feasible, of rearing houses. In the writer's opinion the success of this line of inquiry will largely depend upon further work upon the life-history of the parasite, with special reference to the retention of viability by the spore under Indian climatic conditions, and the mutual relationships of host and parasite.

SUMMARY AND CONCLUSIONS.

(1) Use of the Pasteur method has failed in India to provide a supply of disease-free seed.

(2) This is partly due to ignorant misapplication of the standard method, but largely to its unsuitability, without modification, under Indian conditions.

(3) The wide utilization of multivoltine races in India introduces differences of a fundamental character which render unsuitable a system of examination designed for use on univoltine races in Europe.

(4) A modified method of examination is recommended, based on the observed concentration of spores of the parasite in the gut of the moth.

(5) The rationale of this method and its basis in the mechanism of infection and life-history of the parasite are described.

(6) Owing to the conditions under which sericulture in India is mostly carried on, the provision of disease-free seed of a much higher standard of purity than is necessary in Europe is emphatically required. This is due to the cumulative effect associated with the rearing of the numerous successive broods of the multivoltine races, which leads to rapid progressive increase of the disease in any infected rearing house. Completely disease-free seed can only be secured by the use of a much more critical method of examination of the moth than that at present depended on.

(7) Elimination or reduction in the amount of pebrine in India will depend not only upon stamping out hereditary infection but also upon measures calculated to reduce the incidence of contaminative infection. Further work upon the life-history of the parasite, with special reference to the retention of vitality by the spore, is required to enable practical measures to be devised to deal with this problem.

(8) A hopeful line of enquiry seems to be opened up by the results of some experiments on the increased resistance to infection caused by hill rearing. It seems probable that "ameliorated" seed obtained in this way would constitute a valuable foundation for the introduction of better conditions in the industry. It is also suggested that hybridizing experiments should be carried out with a view to determining the possibilities of raising varieties of hybrids more resistant to infection. This work and that of the production of ameliorated seed of a high grade of purity should be carried out at a central research station; this should be in a suitable situation in the hills and under the control of a biologist.

APPENDIX.

DETAILS OF EXPERIMENTS REFERRED TO IN TEXT.

In all these experiments the controls were reared from part of the same laying or brood as that under treatment.

A.

Artificial infection.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	10 N. caterpillars, 5th stage, were fed with fresh pebrine material. All the meals given to the caterpillars from 25th May to 30th May, 1916, were infected.	11-5-16	15-5-16 18-5-16 21-5-16 24-5-16	30-5-16	8-6-16	One moth of the lot showed pebrine.	
2	10 N. caterpillars, 5th stage. The right side spiracles of the larvæ were painted with fresh pebrine material once every day from 25th to 30th May, 1916.	11-5-16	15-5-16 18-5-16 21-5-16 24-5-16	30-5-16	8-6-16	2 moths of the lot showed pebrine but their air tubes were found to be free from pebrine in paraffin sections.	The gut of the diseased moths showed pebrine. The control of Nos. 1 and 2 had 60 caterpillars which were all found to be disease-free when examined as moths.
3	10 N. caterpillars, 5th stage, were fed with fresh pebrine material. All meals given to the caterpillars from 2nd June to 7th June, 1916, *i.e.*, about 30 meals, were infected.	17-5-16	20-5-16 23-5-16 27-5-16 31-5-16	7-6-16	16-6-16	Negative. 100 per cent. moths were free from the disease.	....
4	10 N. caterpillars, 5th stage. All the spiracles of the caterpillars were painted with fresh pebrine material from 2nd to 7th June, 1916.	....	...	....	....	Ditto	The control of Nos. 3 and 4 had 60 caterpillars which were all found to be disease-free when examined as moths.

EXPERIMENTS 1 to 4.—Pebrine material was derived from diseased moths crushed whole; the experiment was carried out by use of much smaller quantities of infective material than utilized in later experiments. No precautions were taken to prevent action of sunlight or desiccation.

5	10 N. caterpillars, 5th stage, were fed with fresh pebrine material. All meals given to the caterpillars from 15th August to 20th August, 1916, were infected.	2–8–16	5–8–16 8–8–16 11–8–16 14–8–16	20–8–16 and 21–8–16	29–8–16	Positive. 100 per cent. moths showed pebrine.	....
6	10 N. caterpillars, 5th stage. All the spiracles of the caterpillars were painted with fresh pebrine material once daily from 15th August to 20th August, 1916.	2–8–16	5–8–16 8–8–16 11–8–16 14–8–16	20–8–16 and 21–8–16	28–8–16 29–8–16	100 per cent. moths showed pebrine, but their air tubes did not show any attack of pebrine in paraffin sections. Gut of the moths was infected.	The control of Nos. 5 and 6 had 60 caterpillars which were all disease-free when examined as moths.
7	20 N. caterpillars, 4th stage. Fed on fresh pebrine material. All the meals given to the caterpillars from 25th August to 3rd September, 1916, were infected.	16–8–16	19–8–16 22–8–16 25–8–16 28–8–16	3–9–16	13–9–16	100 per cent. moths showed pebrine.	....
8	15 N. caterpillars, 4th stage. All the spiracles of the caterpillars were painted with fresh pebrine once daily from 25th August to 3rd September, 1916.	16–8–16	19–8–16 22–8–16 25–8–16 28–8–16	3–9–16	13–9–16	100 per cent. moths showed pebrine, but their air tubes did not show pebrine in paraffin sections. Gut of the moths was infected.	The control of Nos. 7 and 8 had 90 caterpillars which were all, except one, found to be disease-free when examined as moths.
9	15 N. caterpillars, 5th stage. Fed on fresh pebrine material. All the meals given to the caterpillars from 4th to 9th October, 1916, were infected.	21–9–16	25–9–16 28–9–16 1–10–16 4–10–16	9–10–16	17–10–16	100 per cent. of moths showed pebrine.	....
10	15 N. caterpillars, 5th stage. All the spiracles of the caterpillars were painted with fresh pebrine material once daily from 4th to 9th October, 1916.	21–9–16	25–9–16 28–9–16 1–10–16 4–10–16	9–10–16	17–10–16	100 per cent. moths showed pebrine, but their air tubes did not show any infection in paraffin sections. The gut of the moths was infected.	The control of Nos. 9 and 10 had 90 caterpillars which were found to be disease-free. One specimen in the lot was pebrinized.

Experiments to see if infection is got by rearing normal caterpillars in cages previously smeared with pebrine material.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
11	20 N. caterpillars, 3rd stage, kept and reared till their maturity in a cage, the inside of which was smeared with fresh pebrine material and allowed to dry for a day before use.	16–11–18	23–11–18 29–11–18 6–12–18 14–12–18	31–12–18	22–1–19	Positive. 35 per cent. of moths showed pebrine.	The control had 60 caterpillars. All found to be disease-free on examination as moths.
12	20 N. caterpillars, 4th stage, kept and reared till their maturity in a cage, the inside of which was smeared with fresh pebrine material and allowed to dry for a day before use.	8–11–18	16–11–18 22–11–18 29–11–18 5–12–18	23–12–18	21–1–19	Positive. 44 per cent. of moths showed pebrine.	The control had 60 caterpillars. All found to be disease-free when examined as moths.

Experiments to see if the fæces of pebrinized larvæ are infective.

13	80 N. caterpillars, 3rd stage, were fed at all meals after 2nd moult on leaf smeared with fæces of diseased larvæ. Examination of these fæces showed that they contained pebrine spores.	27–6–16	30–6–16 3–7–16 6–7–16 10–7–16 11–7–16	17–7–16	....	Positive. Infection became apparent in caterpillars from 11th July, 1916. All pupæ failed to emerge as moths. All were pebrinized.	Control. 80 caterpillars. All disease-free when examined as moths.
14	10 N. caterpillars, 4th stage. Once every day after 3rd moult, fresh fæces containing pebrine spores, obtained from a diseased caterpillar, were sprinkled over the caterpillars and the leaf served to them.	2–8–16	5–8–16 8–8–16 11–8–16 14–8–16	20–8–16 and 21–8–16	28–8–16 and 29–8–16	Positive. 100 per cent. of moths showed pebrine.	The control had 60 caterpillars. All found to be free from disease when examined as moths.

B.

Experiments to see whether diseased male moth if allowed to couple with normal female moth would transmit disease to the progeny.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of of cocoons	Date of emergence of moth	Results	Remarks
1	Eggs laid by a N. female moth which had been allowed to couple with a diseased male moth were hatched and reared.	4–4–19	10–4–19 14–4–19 18–4–19 23–4–19	1–5–19 and 2–5–19	10–5–19 and 11–5–19	Negative. 100 per cent. of moths free from pebrine.	....
2	Ditto. ditto. ..	4–4–19	10–4–19 14–4–19 18–4–19 23–4–19	1–5–19 and 2–5–19	10–5–19 and 11–5–19	Negative. 100 per cent. of moths free from pebrine.	....
3	Progeny of a N. female moth coupled with a diseased male moth.	27–6–19	1–7–19 4–7–19 7–7–19 10–7–19	16–7–19 and 17–7–19	25–7–19 and 26–7–19	20 per cent. of the moths showed pebrine.	....
4	Ditto. ditto. ..	27–6–19	1–7–19 4–7–19 7–7–19 10–7–19	16–7–19 and 17–7–19	25–7–19 and 26–7–19	9·9 per cent. of the moths showed pebrine.	....
5	Ditto. ditto ..	27–6–19	1–7–19 4–7–19 7–7–19 10–7–19	16–7–19 and 17–7–19	25–7–19 and 26–7–19	10·7 per cent. of the moths showed pebrine.	....

C.

Effect of artificial infection after first moult.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	15 N. caterpillars. 2nd stage. Fed on leaf smeared with fresh pebrine material. All meals given to the caterpillars after the first moult were infected.	16-8-16	19-8-16 22-8-16 25-8-16 28-8-16 29-8-16	3-9-16 and 4-9-16	13-9-16 and 14-9-16	Positive. 100 per cent. moths were pebrinized. Infection found in dead caterpillars from the 1st September, 1916, 2 days before spinning.	The control had 90 caterpillars, one out of which was found pebrinized and the rest disease-free.
2	70 N. caterpillars. 2nd stage. Fed on leaf smeared with fresh pebrine material. All meals given to the caterpillars after the first moult were infected.	29-10-16	1-11-16 5-11-16 10-11-16 14-11-16 15-11-16	24-11-16 25-11-16 and 26-11-16	18-12-16 and 19-12-16	Positive. 100 per cent. moths showed pebrine. Infection found on examination in caterpillars from 21st November, 1916, 3 days before spinning.	....
3	50 N. caterpillars, 2nd stage. From 1st November to 28th November, 1916, fed one meal daily with leaf infected with fresh pebrine material. Normal leaf given to caterpillars at other meals.	29-10-16	1-11-16 5-11-16 10-11-16 14-11-16	27-11-16 and 28-11-16	18-12-16 and 19-12-16	Positive. 100 per cent. of moths showed pebrine. Infection found on examination in caterpillars from 29th November, 1916, *i.e.*, caterpillars failed to spin.	The control of Nos. 2 and 3 had 90 caterpillars, one out of which was found to be pebrinized and the rest disease-free.

4	30 N. caterpillars, 2nd stage. Fed on leaf smeared with fresh pebrine material at only the first meal after every moult.	21–9–16	25–9–16 28–9–16 1–10–16 4–10–16	9–10–16 and 10–10–16	20–10–16 and 21–10–16	Positive. 100 per cent. of moths showed pebrine. Infection was found on examination in larvæ from 2nd October, 1916, 7 days before spinning.	The control had 90 caterpillars. All found to be disease-free on examination as moths.
5	40 N. caterpillars, 2nd stage. Fed on leaf smeared with fresh pebrine materials at only the first meal after every moult.	29–10–16	1–11–16 5–11–16 10–11–16 15–11–16	26–11–16 and 27–11–16	18–12–16 and 19–12–16	Positive. 100 per cent. of moths showed pebrine. Infection found on examination in larvæ from 20th November, 1916, 7 days before spinning.	The control of this experiment was the same as the one for Nos. 2 and 3.

D.

Effect of artificial infection after second moult.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	22 N. caterpillars, 3rd stage. Fed on leaf smeared with fresh pebrine material at all meals from 14th to 27th June, 1916	7-6-16	10-6-16 13-6-16 17-6-16 20-6-16	27-6-16	6-7-16	Positive. 100 per cent. of moths showed pebrine. Infection found on examination in pupæ from 1st July, 1916.	The control had 22 caterpillars. All found to be disease-free on examination as moths.
2	40 N. caterpillars, 3rd stage. Fed on leaf smeared with fresh pebrine material at all meals after 2nd moult.	28-10-16	1-11-16 4-11-16 9-11-16 14-11-16	25-11-16 and 26-11-16	18-12-16 and 19-12-16	Positive. 100 per cent. of moths showed pebrine. Infection found on examination in larvæ from 22nd November, 1916, 3 days before spinning.	The control had 90 caterpillars. Two moths showed pebrine and the rest were disease-free.
3	45 N. caterpillars, 3rd stage. Fed on leaf smeared with fresh pebrine material only once after 2nd moult. Other meals consisted of normal leaf.	29-3-18	4-4-18 8-4-18 12-4-18 16-4-18 17-4-18	25-4-18 and 26-4-18	4-5-18 and 5-5-18	Positive. 87·8 per cent. of moths showed pebrine. Infection apparent from 26th April, 1918, in caterpillars that failed to spin and died.	The control had 90 caterpillars. All found to be disease-free on examination as moths.
4	45 N. caterpillars, 3rd stage. Fed on leaf smeared with fresh pebrine material, three	29-3-18	4-4-18 8-4-18 12-4-18	25-4-18 and 26-4-18	4-5-18 and 5-5-18	Positive. 70 per cent. of moths showed pebrine. Infection found from	The control had 90 caterpillars. All found to be disease-free when

	meals after 2nd moult between 11th and 14th April, 1918.		16–4–18			29th April, 1918, in dead pupæ.	examined as moths.
5	60 N. caterpillars, 3rd stage. Fed on leaf smeared with fresh pebrine material for one meal after 2nd moult. Other meals were uninfected.	12–5–18	16–5–18 19–5–18 22–6–18 25–5–18	31–5–18 and 3–6–18	11–6–18 and 12–6–18	Positive. 85·7 per cent. of moths showed pebrine. Infection found from 30th May, 1918, in dead larvæ.	The control had 90 caterpillars. All found to be disease-free on examination as moths.
6	30 N. caterpillars, 3rd stage. Fed on leaf smeared with fresh pebrine material, two meals on 23rd June, 1918. Other meals of uninfected leaf.	17–6–18	20–6–18 23–6–18 26–6–18 29–6–18	4–7–18	11–7–18 and 12–7–18	Positive. 100 per cent. moths showed pebrine. Infection found in caterpillars from 30th June, 1918.	The control had 90 caterpillars. All found to be disease-free on examination as moths.
7	80 N. caterpillars, 3rd stage, were fed at all meals after 2nd moult on leaf smeared with fæces of diseased larvæ. Examination of these fæces showed them to contain pebrine spores.	27–6–16	30–6–16 3–7–16 6–7–16 10–7–16 11–7–16	17–7–16	....	Positive. Infection found in larvæ from 11th July, 1916. All pupæ failed to emerge as moth. All were pebrinized.	The control had 80 caterpillars. All found to be disease-free when examined as moths.
8	20 N. caterpillars, 3rd stage, were kept and reared till their maturity in a cage, the inside of which was smeared with fresh pebrine material and allowed to dry for a day before use.	16–11–18	23–11–18 29–11–18 6–12–18 14–12–18	31–12–18	22–1–19	Positive. 35 per cent. of moths showed pebrine.	The control had 60 caterpillars. All found to be disease-free when examined as moths.

E.

Effect of artificial infection after third moult.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	30 N. caterpillars, 4th stage. Fed on leaf smeared with fresh pebrine material, one meal daily from 18th November to 25th November, 1917.	3–11–17	8–11–17 12–11–17 17–11–17 26–11–17	7–12–17 and 8–12–17	2–1–18 and 3–1–18	Positive. 100 per cent. of moths showed pebrine. Infection found in larvæ from 20th November, 1917.	The control had 90 caterpillars. All found to be disease-free on examination as moths.
2	30 N. caterpillars, 4th stage. Fed on leaf smeared with fresh pebrine material, one meal daily from 21st to 27th February, 1918.	24–1–18	4–2–18 14–2–18 21–2–18 27–2–18	8–3–18	19–3–18 and 20–3–18	Positive. 100 per cent. of the moths showed pebrine. Infection found in larvæ on 3rd March, 1918.	The control had 60 caterpillars. All found to be disease-free on examination as moths.
3	40 N. caterpillars, 4th stage. Fed on leaf smeared with fresh pebrine material, four meals after 3rd moult. Other meals of uninfected leaf.	16–11–18	23–11–18 29–11–18 5–12–18 14–12–18	31–12–18	22–1–19	Positive. 59 per cent. of moths showed pebrine.	The control had 60 caterpillars. All found to be disease-free on examination as moths.
4	30 N. caterpillars, 4th stage. Fed on leaf smeared with fresh pebrine materials, two meals after 3rd moult, other meals of uninfected leaf.	28–5–18	31–5–18 3–6–18 6–6–18 10–6–18	17–6–18 to 20–6–18	....	Positive. 100 per cent. of the caterpillars and pupæ that were examined were pebrinized. No moth emerged. Infection found in caterpillars from 20th June, 1918.	The control had 90 caterpillars. All found to be disease-free on examination as moths.

5	20 N. caterpillars, 4th stage. Fed on leaf smeared with fresh pebrine material, all meals from 25th August to 3rd September, 1916.	16–8–16	19–8–16 22–8–16 25–8–16 28–8–16	3–9–16	13–9–16	Positive. 100 per cent. of moths showed pebrine.	The control had 90 caterpillars. All found to be free from pebrine when examined as moths.
6	10 N. caterpillars, 4th stage. Kept with the excreta of diseased caterpillars. Every day fresh diseased excreta were put in the cage (after the 3rd moult).	2–8–16	5–8–16 8–8–16 11–8–16 14–8–16	20–8–16 and 21–8–16	28–8–16 and 29–8–16	Positive. 100 per cent. of moths showed pebrine. Infection not found in larvæ.	The control had 60 caterpillars. All found to be disease-free when examined as moths.
7	20 N. caterpillars, 4th stage, were kept and reared till their maturity in a cage, the inside of which was smeared with fresh pebrine material and allowed to dry for a day before use.	8–11–18	16–11–18 22–11–18 29–11–18 5–12–18	23–12–18	21–1–19	Positive. 44 per cent. of moths showed pebrine.	The control had 60 caterpillars. All found to be disease-free on examination as moths.

F.

Effect of artificial infection after fourth moult.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of of cocoons	Date of emergence of moth	Results	Remarks
1	10 N. caterpillars, 5th stage. Fed on leaf smeared with fresh pebrine material, all meals from 25th to 30th May, 1916.	11-5-16	15-5-16 18-5-16 21-5-16 24-5-16	30-5-16	8-6-16	10 per cent. moths showed pebrine.	The control had 60 caterpillars. All found to be free from pebrine when examined as moths.
2	10 N. caterpillars, 5th stage. Fed on leaf smeared with fresh pebrine material at all meals from 2nd to 7th June, 1916.	17-5-16	20-5-16 23-5-16 27-5-16 31-5-16	7-6-16	16-6-16	Negative. 100 per cent. moths free from pebrine.	Ditto.
3	15 N. caterpillars, 5th stage. Fed on leaf smeared with fresh pebrine material, all meals from 4th to 9th October, 1916.	21-9-16	25-9-16 28-9-16 1-10-16 4-10-16	9-10-16	17-10-16	Positive. 100 per cent. moths showed pebrine.	The control had 90 caterpillars. All found to be free from pebrine when examined as moths.
4	10 N. caterpillars, 5th stage. Fed on leaf smeared with fresh pebrine material, all the meals after 4th moult.	2-8-16	5-8-16 8-8-16 11-8-16 14-8-16	21-8-16	29-8-16	Positive. 100 per cent. of moths showed pebrine. Infection found in larvæ from 24th August, 1916.	The control had 60 caterpillars. All found to be disease-free when examined as moths.

5	30 N. caterpillars, 5th stage. Fed on leaf smeared with fresh pebrine material, one meal daily after the fourth moult. Other meals of uninfected leaf.	28–10–16	1–11–16 4–11–16 9–11–16 14–11–16	27–11–16 and 28–11–16	18–12–16	Positive. 37·5 per cent. of moths showed pebrine. Infection found in larvæ.	The control had 50 caterpillars, 2 moths of the lot showed pebrine and the rest free from pebrine.
6	Ditto ..	31–3–17	6–4–17 12–4–17 17–4–17 22–4–17	29–4–17	8–5–17	Positive. 100 per cent. of moths showed pebrine. Infection not found in larvæ.	The control had 90 caterpillars, which were all found to be disease-free on examination as moths.
7	40 N. caterpillars, 5th stage. Fed on leaf smeared with fresh pebrine material, four meals. Other meals of uninfected leaf.	8–11–18	16–11–18 22–11–18 28–11–18 4–12–18	23–12–18 and 24–12–18	22–1–19 and 23–1–19	Ditto	The control had 60 caterpillars. All found to be disease-free on examination as moths.
8	30 N. caterpillars, 4th stage. Fed for four meals on leaf smeared with fresh pebrine material (one every day from 7th to 10th September, 1918). Other meals of uninfected leaf. (*Hill rearing.*)	....	3rd moult 7–9–18 4th moult 18–9–18	1–10–18	19–10–18	94 per cent. of the moths showed pebrine.	The control had 90 caterpillars. All found to be free from pebrine.

G.

Effect of artificial infection a day before spinning.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of *moth*	Results	Remarks
1	30 N. caterpillars, 5th stage. Given one meal of leaf smeared with fresh pebrine material just a day before spinning cocoons. Other meals of uninfected leaf.	17–6–18	20–6–18 23–6–18 25–6–18 28–6–18	4–7–18	11–7–18 and 12–7–18	Positive. 74 per cent. of moths showed pebrine. The infection was very mild and could be seen in gut but not in colon content, etc. Infection did not become apparent in pupæ.	The control had 60 caterpillars. All found to be disease-free when examined as moths.
2	15 N. caterpillars, 5th stage. Given one meal of leaf smeared with fresh pebrine material just a day before spinning cocoons. Other meals of uninfected leaf.	16–2–19	27–2–19 5–3–19 12–3–19 20–3–19	30–3–19	11–4–19	Positive. 63·5 per cent. of the moths showed pebrine. Infection did not become apparent in pupæ. Infection in moth was very mild.	The control had 90 caterpillars. All found to be disease-free when examined as moths.
3	15 N. caterpillars, 5th stage. Given one meal of fresh pebrine material just a day before spinning of cocoons.	2–4–19	8–4–19 13–4–19 17–4–19 22–4–19	1–5–19	10–5–19	Positive. 38·5 per cent. of the moths showed pebrine. Infection in pupæ was noticed in two cases out of five by paraffin section.	The control had 60 caterpillars. All found to be disease-free when examined as moths.

4	30 N. caterpillars, 5th stage. Given one meal of fresh pebrine material just a day before spinning on 7th September, 1918. (*Hill rearing.*)	....	....	8–9–18	23–9–18	40 per cent. of the moths showed pebrine. Very mild attack had taken place.	The control had 90 caterpillars. All found to be free from pebrine when examined as moths.

H.

Experiments on infection through contaminated eggs.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	REMARKS
1	One N. laying of eggs was dusted with fresh powdered pebrine material, three days before hatching, and was allowed to hatch out unwashed. Entire leaf was spread over the larvæ at the time of their hatching for removing them from the paper.	3-3-19	11-3-19 17-3-19 23-3-19 30-3-19	8-4-19 and 9-4-19	17-4-19 and 18-4-19	One moth out of 49 showed pebrine.	....
2	One N. laying of eggs was dusted with powdered fresh pebrine material, three days before hatching. Two days after dusting it was washed with water for 2 minutes. Entire leaf was spread over the caterpillars at the time of their hatching for removing them from the paper.	3-3-19	11-3-19 17-3-19 22-3-19 30-3-19	8-4-19 and 9-4-19	17-4-19 and 18-4-19	Negative. 100 per cent. of moths free from pebrine.	....
3	One N. laying of eggs was dusted with powdered fresh pebrine material, three days before hatching. Two days after the laying was dusted. Was washed with 1 per cent. formalin solution for 1 minute and then washed with water for 2 minutes. Entire leaf was spread over the	3-3-19	11-3-19 17-3-19 22-3-19 23-3-19	8-4-19 and 9-4-19	17-4-19 and 18-4-19	Negative. 100 per cent. of moths disease-free.	....

	larvæ at the time of their hatching for removing them from the paper.						
4	Half of a N. laying which had been dusted with fresh pebrine spores on 24th June, 1919. Washed the laying with 1 per cent. formalin solution for 1 minute and with water for 2 minutes on 26th June, 1919. Chopped up leaf given at the first meal. Half of the above N. laying which had been dusted with fresh pebrine spores on 24th June, 1919. Allowed to hatch out; unwashed chopped up leaf given at the first meal.	27–6–19	30–6–19 3–7–19 6–7–19 9–7–19	15–7–19 and 16–7–19	23–7–19 and 24–7–19	Negative. 100 per cent. of the moths free from pebrine.	....
5	Half a N. laying which had been dusted with fresh pebrine spores on 24th June, 1919. Washed the laying with water for 2 minutes on 26th June, 1919. Chopped up leaf given at the first meal. Half of the above N. laying which was dusted with fresh pebrine spores on 24th June, 1919. Allowed to hatch out unwashed. Entire leaf given at the first meal.	27–6–19	30–6–19 3–7–19 6–7–19 9–7–19	15–7–19 and 16–7–19	23–7–19 and 24–7–19	Ditto	....
6	One N. laying which had been dusted with fresh pebrine spores on 24th June, 1919. Allowed to hatch out unwashed. Entire leaf given at the first meal.	27–6–19	30–6–19 3–7–19 6–7–19 9–7–19	15–7–19 and 16–7–19	23–7–19 and 24–7–19	Ditto	....

K.

Experiments to determine the viability of pebrine spores under different conditions of moisture.

Method of keeping pebrine material moist :—Thick watery suspension pebrine spores taken up with blotting paper and kept in bell jar on a glass bridge. The bell jar contained water to prevent drying up of the blotting paper.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	30 N. caterpillars, 2nd stage. Fed on leaf smeared with pebrine material which had been kept moist for 6½ months from 24th September, 1916, to 11th April, 1917. One meal daily of this material was given to the caterpillars after the first moult.	5-4-17	11-4-17 16-4-17 21-4-17 27-4-17	4-5-17	15-5-17	Negative. 100 per cent. moths free from pebrine.	The control had 60 caterpillars. All disease-free when examined as moths.
2	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material which had been kept moist for 4 weeks. One infected meal daily was given to the caterpillars.	16-5-17	20-5-17 23-5-17 26-5-17 29-5-17	4-6-17 and 5-6-17	14-6-17 and 15-6-17	Negative. 100 per cent. moths free from pebrine.	The control had 60 caterpillars. All disease-free when examined as moths.
3	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material which had been kept moist for 8 months from 24th September, 1916, to 25th May, 1917. One infected meal daily given to the caterpillars after the 2nd moult.	17-5-17	21-5-17 24-5-17 27-5-17 30-5-17	5-6-17 and 6-6-17	15-6-17	Ditto	The control had 60 caterpillars. All found to be free from disease when examined as moths.

4	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material which had been kept moist for 9¾ weeks from 23rd April to 29th June, 1917. One infected meal daily given to the caterpillars after the 2nd moult.	23–6–17	26–6–17 29–6–17 2–7–17 6–7–17	12–7–17 and 13–7–17	21–7–17 and 22–7–17	Negative. 100 per cent. of moths free from disease.	The control had 60 caterpillars. All found to be disease-free when examined as moths.
5	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material which had been kept moist from November, 1916, to 23rd July, 1917, *i.e.*, 8¾ months. Two infected meals daily given to the caterpillars after the 2nd moult.	15–7–17	19–7–17 22–7–17 25–7–17 29–7–17	3–8–17	13–8–17	Negative. 100 per cent. moths disease-free.	The control had 90 caterpillar. All found to be disease-free when examined as moths.
6	30 N. caterpillars, 3rd stage. Fed on pebrine material which had been kept moist from 23rd April to 23rd July, 1917, *i.e.*, 3 months. Two infected meals were given daily to caterpillars after the second moult.	15–7–17	19–7–17 22–7–17 25–7–17 29–7–17	2–8–17 and 3–8–17	13–8–17	Negative. 100 per cent. of moths free from *pebrine*.	The control had 90 caterpillars. All found to be free from pebrine when examined as moths.
7	30 N. caterpillars, 5th stage. Fed on leaf smeared with pebrine material which had been kept moist for 5 months from 26th June to 26th November, 1917. One meal daily of infected material was given to the caterpillars after the 4th moult.	3–11–17	8–11–17 12–11–17 17–11–17 25–11–17	4–12–17	28–12–17	*Ditto*	*Ditto*

Experiments to determine the viability of pebrine spores under different conditions of moisture.—contd.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
8	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material which had been kept moist for 8½ months from 26th June, 1917, to 15th March, 1918. One infected meal daily was given to the caterpillars from 15th to 30th March, 1918.	2-3-18	7-3-18 12-3-18 17-3-18 22-3-18	30-3-18 and 31-3-18	11-4-18	Negative. 100 per cent. of moths free from disease.	The control had 90 caterpillars, which were found to be disease-free when examined as moths.
9	60 N. caterpillars, 4th stage. Fed on leaf smeared with pebrine material which had been kept moist from 26th April to 6th June, 1918, *i.e.*, 6 weeks. One infected meal daily given to the caterpillars after 3rd moult.	28-5-18	31-5-18 3-6-18 6-6-18 10-6-18	16-6-18 and 17-6-18	25-6-18	Out of 48 moths one was found to be pebrinized.	The control had 60 caterpillars. All found to be free from pebrine when examined as moths.
10	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material which had been kept moist from 26th April to 23rd June, 1918, *i.e.*, 7½ months. One infected meal daily given to the caterpillars after the 2nd moult.	4-6-18	20-6-18 23-6-18 26-6-18 29-6-18	4-7-18	11-7-18 and 12-7-18	Negative. 100 per cent. of moths free from pebrine.	The control had 90 caterpillars. All found to be free from pebrine when examined as moths.
11	50 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material kept in a culture pot on earth for 5½ weeks from 15th June to 26th July, 1918. The	20-7-18	23-7-18 26-7-18 29-7-18 1-8-18	6-8-18	16-8-18	Ditto	The control had 80 caterpillars. All found to be disease-free when examined as moths.

	earth in the culture pot was kept moist. One infected meal daily was given after the 2nd moult.						
12	30 N. caterpillars, 2nd stage. Fed on leaf smeared with pebrine material kept in a desiccator from 25th October, 1916, to 9th April, 1917, *i.e.*, 5½ months. One meal daily after the 1st moult was infected.	3–4–17	9–4–17 15–4–17 20–4–17 26–4–17	3–5–17 and 4–5–17	15–5–17	Positive. 20 per cent. of moths pebrinized.	The control had 60 caterpillars. All found to be disease-free when examined as moths.
13	30 N. caterpillars, 2nd stage. Fed on leaf smeared with pebrine material kept dry in a desiccator from 25th October, 1916, to 11th April, 1917, *i.e.*, 5½ months. One infected meal daily was given after the first moult.	5–4–17	11–4–17 16–4–17 21–4–17 27–4–17	4–5–17 and 5–5–17	15–5–17 and 16–5–17	Positive. 44·4 per cent. of moths showed pebrine.	Ditto
14	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material kept dry in a desiccator for 4⅔ months from 5th January to 24th May, 1917. Given one infected meal daily after 2nd moult.	17–5–17	21–5–17 24–5–17 27–5–17 30–5–17	5–6–17 and 6–6–17	15–6–17	Negative. 100 per cent. of the moths free from pebrine.	The control had 60 caterpillars. All found to be free from pebrine when examined as moths.
15	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material kept dry in a desiccator from 22nd December, 1916, to 29th June, 1917, *i.e.*, 6½ months. Given one infected meal daily after 2nd moult.	29–6–17	26–6–17 29–6–17 2–7–17 6–7–17	12–7–17 and 13–7–17	21–7–17 and 22–7–17	Negative. 100 per cent. moths free from pebrine.	The control had 60 caterpillars. All found to be free from pebrine when examined as moths.
16	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material kept dry in a petri dish from July, 1916, to 4th August, 1917, *i.e.*, 13 months. Given one infected meal daily after 2nd moult.	29–7–17	1–8–17 4–8–17 7–8–17 11–8–17	18–8–17	26–8–17	Negative. 100 per cent. of moths free from pebrine, but they all showed attack of flacherie.	The control had 90 caterpillars. All found to be free from disease when examined as moths.

Experiments to determine the viability of pebrine spores under different conditions of moisture.—contd.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
17	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material kept dry in powdered condition in a petri dish from November 1916 to 4th August, 1917, *i.e.*, 9 months. Given one infected meal daily after 2nd moult.	29-7-17	1-8-17 4-8-17 7-8-17 11-8-17	18-8-17	26-8-17	Negative. 100 per cent. of moths free from pebrine, but all showed attack of flacherie.	The control had 90 caterpillars. All found to be disease-free when examined as moths.
18	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material kept dry in a desiccator for 8½ months from June 1917 to 15th March, 1918. Given one infected meal daily from 15th March to 30th March, 1918.	2-3-18	7-3-18 12-3-18 17-3-18 22-3-18	30-3-18 and 31-3-18	10-4-18 and 11-4-18	Negative. 100 per cent. of moths free from pebrine.	The control had 90 caterpillars. All found to be disease-free when examined as moths.
19	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material kept dry in a desiccator from January 1917 to 16th March, 1918, *i.e.*, 14½ months. Given one meal (infected) daily after the 2nd moult.	4-3-18	10-3-18 15-3-18 20-3-18 25-3-18	2-4-18 and 3-4-18	15-4-18	Negative. 100 per cent. of moths free from pebrine.	The control had 90 caterpillars. All found to be free from disease when examined as moths.
20	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material kept dry in a desiccator from 10th December, 1917, to 23rd June, 1918, *i.e.*, 6 months 3 days.	17-6-18	20-6-18 23-6-18 26-6-18 29-6-18	4-7-18	11-7-18 and 12-7-18	Negative. 100 per cent. of moths free from disease.	The control had 90 caterpillars. All found to be free from disease when examined as moths.

	Given one infected meal daily after the 2nd moult.						
21	30 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material kept dry in a desiccator from 10th December, 1917, to 23rd June, 1918, *i.e.*, 6½ months. Given one infected meal daily after the 2nd moult.	3–7–18	6–7–18 9–7–18 13–7–18 16–7–18	21–7–18	1–8–18	Negative. 100 per cent. of moths free from pebrine.	The control had 90 caterpillars. All found to be disease-free when examined as moths.
22	50 N. caterpillars, 3rd stage. Fed on leaf smeared with pebrine material kept dry in a desiccator from March 1918 to 26th July, 1918, *i.e.*, 5 months. Given one infected meal daily after the 2nd moult.	20–7–18	23–7–18 26–7–18 29–7–18 1–8–18	4–8–18	16–8–18	One moth out of 43 showed pebrine.	The control had 80 caterpillars. All found to be disease-free on examination as moths.
23	20 N. caterpillars, 5th stage. Fed on leaf smeared with pebrine material kept dry in a desiccator from 15th April to 23rd May, 1919, *i.e.*, 5 weeks. One infected meal given on 23rd May, 1919, and another feed on 25th May, 1919.	29–4–19	4–5–19 9–5–19 13–5–19 17–5–19	27–5–19	5–6–19 and 6–6–19	Positive. 30 per cent. of moths showed pebrine.	The control had 60 caterpillars. All found to be disease-free on examination as moths.
24	30 N. caterpillars, 5th stage. Fed 2 meals of pebrine material kept dry in a desiccator from 15th July, 1918, to 25th August, 1918, *i.e.*, 5½ weeks. Infected meals given on 7th September, 1918.	....	....	8–9–18	23–9–18	Negative. 100 per cent. of the moths free from pebrine.	The control had 90 caterpillars. All found to be free from pebrine.
25	20 N. caterpillars, 5th stage. Fed on two meals smeared with pebrine material which has been kept in a desiccator for 2½ months, *i.e.*, 15th April, 1919, to 3rd July, 1919. Given infected meal on 3rd July, 1919.	13–6–19	17–6–19 21–6–19 24–6–19 28–6–19	5–7–19	13–7–19	10 per cent. of the moths showed pebrine.	The control had 40 caterpillars. All found to be disease-free.

L.

Experiments on effect of environment upon resistance to infection.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	Half of a diseased moth's laying reared under better sanitary conditions and given more meals and more space than the—	19–5–17	24–5–17 27–5–17 30–5–17 3–6–17	10–6–17	19–6–17 and 20–6–17	100 per cent. of the moths were pebrinized. Weight of cocoons, 2 gm. = 24 cocoons without chrysalis.	....
	—Other half which was reared under ordinary conditions.	19–5–17	24–5–17 26–5–17 27–5–17 31–5–17 4–6–17	11–6–17 and 12–6–17	..	No moths emerged. All pupæ were dead and heavily pebrinized. Weight of cocoons, 2 gm. = 38 cocoons without chrysalis.	
2	Half of a diseased moth's laying reared under better sanitary conditions and given more meals and more space than the—	1–7–17	9–7–17 12–7–17 16–7–17 20–7–17	27–7–17	8–8–17	10·3 per cent. moths showed pebrine. Weight of cocoons, 2 gm. = 18 cocoons without chrysalis.	....
	—Other half of the diseased laying which was reared under ordinary conditions.	1–7–17	9–7–17 12–7–17 16–7–17 20–7–17	28–7–17 and 29–7–17	8–8–17	20·3 per cent. of moths showed pebrine. Weight of cocoons, 2 gm. = 22 cocoons without chrysalis.	
3	Half of a diseased moth's laying reared under better sanitary conditions and given more meals and more space than the—	16–7–17	20–7–17 24–7–17 28–7–17 1–8–17	10–8–17 and 11–8–17	20–8–17	100 per cent. moths showed pebrine. Weight of cocoons, 2 gm. = 21 cocoons without chrysalis.	....
	—Other half of the same diseased laying which was reared under ordinary conditions.	16–7–17	20–7–17 25–7–17 29–7–17 2–8–17	11–8–17 and 12–8–17	....	No moths emerged. All pupæ died and were pebrinized. Weight of cocoons, 2 gm. = 32 cocoons without chrysalis.	

4	Half of a diseased moth's laying reared under better sanitary conditions and given more meals and more space than the—	4–8–17	7–8–17 10–8–17 13–8–17 16–8–17	23–8–17	30–8–17
	—Other half of the same diseased laying which was reared under ordinary conditions.	4–8–17	7–8–17 10–8–17 13–8–17 16–8–17	23–8–17	30–8–17
5	Half of a diseased moth's laying reared under better sanitary conditions, and given more meals and more space than the—	14–11–17	21–11–17 25–11–17 3–12–17 15–12–17	22–12–17	6–1–18
	—Other half of the same diseased laying which was reared under ordinary conditions.	14–11–17	21–11–17 25–11–17 3–12–17 6–12–17 14–12–17	22–12–17 and 23–12–17	8–1–18
6	Half of a diseased moth's laying reared under better sanitary conditions and given more meals and more space than the	14–11–17	21–11–17 27–11–17 3–12–17 13–12–17	22–12–17	6–1–18
	—Other half of the same diseased laying which was reared under ordinary conditions.	14–11–17	21–11–17 27–11–17 3–12–17 14–12–17	22–12–17 and 23–12–17	6–1–18 and 7–1–18
7	Half of a diseased moth's laying reared under better sanitary conditions, given more meals and more space than the—	14–11–17	21–11–17 27–11–17 3–12–17 14–12–17	22–12–17	6–1–18
	—Other half of the same diseased laying which was reared under ordinary conditions.	14–11–17	21–11–17 27–11–17 3–12–17 6–12–17 14–12–17	23–12–17	7–1–18

4	Half of a diseased moth's laying reared under better sanitary conditions and given more meals and more space than the—	100 per cent. of moths showed pebrine. Weight of cocoons, 2 gm. = 20 cocoons without chrysalis.
	—Other half of the same diseased laying which was reared under ordinary conditions.	100 per cent. of moths showed pebrine. Weight of cocoons, 2 gm. = 30 cocoons without chrysalis.
5	Half of a diseased moth's laying reared under better sanitary conditions, and given more meals and more space than the—	44·7 per cent. of moths showed pebrine. Weight of cocoons, 2 gm. = 23 cocoons without chrysalis.
	—Other half of the same diseased laying which was reared under ordinary conditions.	52·6 per cent. of moths showed pebrine. Weight of cocoons, 2 gm. = 38 cocoons without chrysalis.
6	Half of a diseased moth's laying reared under better sanitary conditions and given more meals and more space than the	31·7 per cent. of moths showed pebrine. Weight of cocoons, 2 gm. = 17 cocoons without chrysalis.
	—Other half of the same diseased laying which was reared under ordinary conditions.	28·1 per cent. of moths showed pebrine. Weight of cocoons, 2 gm. = 23 cocoons without chrysalis.
7	Half of a diseased moth's laying reared under better sanitary conditions, given more meals and more space than the—	39·4 per cent. of moths showed pebrine. Weight of cocoons, 2 gm. = 19 cocoons without chrysalis.
	—Other half of the same diseased laying which was reared under ordinary conditions.	62·4 per cent. of moths showed pebrine. Weight of cocoons, 2 gm. = 25 cocoons without chrysalis.

M.

Experiments to test the comparative destructive effect on pebrine spores of 1 per cent. formalin solution, and 1 per cent. copper sulphate solution and hypochlorite solution.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	30 N. caterpillars, 4th stage. Fed on fresh pebrine material which had been treated with 1 per cent. formalin solution. One meal of treated pebrinized material given daily from 18th to 25th November, 1917.	3-11-17	8-11-17 12-11-17 17-11-17 26-11-17	7-12-17 and 8-12-17	1-1-18 and 2-1-18	100 per cent. of the moths free from pebrine.	The control had 60 caterpillars. All found to be disease-free on examination as moths.
2	30 N. caterpillars, 4th stage. Fed on fresh pebrine material which had been treated with 1 per cent. copper sulphate solution. One meal of treated pebrinized material given daily from 18th to 25th November, 1917.	3-11-17	8-11-17 12-11-17 17-11-17 26-11-17	7-12-17 and 8-12-17	1-1-18 2-1-18 and 3-1-18	100 per cent. of the moths showed pebrine.	The control had 90 caterpillars. All found to be disease-free when examined as moths.

Experiments to see the effect of dilute hypochlorite solution (E. C.) on pebrine spores.

3	25 N. caterpillars, 4th stage. Fed at one meal leaf smeared with fresh pebrine material which had been treated for	3-3-19	11-3-19 17-3-19 22-3-19 29-3-19	8-4-19 and 9-4-19	18-4-19 and 19-4-19	100 per cent. of the moths were free from pebrine.	The control had 32 caterpillars. All found to be disease-free. The pebrine material which

	about 5 minutes with 3 per cent. of E.C. containing 2·75 per cent. Cl_2 ; on 23rd March, 1919.						was used was found to be infective before treatment with E.C.
4	20 N. caterpillars, 5th stage. Fed at one meal leaf smeared with fresh pebrine material which had been treated for about 5 minutes with 3 per cent. of E.C. containing 2·2 per cent. Cl_2 ; on 30th April, 1919.	2–4–19	8–4–19 13–4–19 17–4–19 22–4–19	1–5–19	10–5–19	100 per cent. of the moths were free from pebrine.	The control had 120 caterpillars. All found to be disease-free. The pebrine material which was used was found to be infective before treatment with E.C.
5	20 N. caterpillars, 5th stage. Fed at two meals leaf smeared with fresh pebrine material which had been treated for about 5 minutes with 3 per cent. of E.C. containing 2·7 per cent. Cl_2. One infected meal was given on 23rd May and the other on 25th May, 1919.	29–4–19	1–5–19 9–5–19 13–5–19 17–5–19	27–5–19	5–6–19 and 6–6–19	31·6 per cent. of the moths showed pebrine.	The control had 80 caterpillars. All found to be disease-free.
6	20 N. caterpillars, 5th stage. Fed at one meal leaf smeared with fresh pebrine material which had been treated for about 5 minutes with 3 per cent. of E.C. containing 2·2 per cent. Cl_2 on 3rd July, 1919.	13–6–19	17–6–19 21–6–19 24–6–19 28–6–19	5–7–19	13–7–19	100 per cent. of the moths were free from pebrine.	The control had 40 caterpillars. All found to be disease-free. The pebrine material used was found to be infective before treatment with E.C.

N.

Experiments to see the effect of direct sun's rays on pebrine spores.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	20 N. caterpillars, 4th stage. Fed one meal of fresh pebrine material which had been dried in direct sun's rays in a glass vessel for 2 days. Fed on 15th March, 1919.	18–2–19	27–2–19 5–3–19 12–3–19 20–3–19	29–3–19 and 30–3–19	9–4–19	One moth out of 13 moths showed pebrine.	The control had 60 caterpillars. All found to be disease-free when examined as moths.
2	20 N. caterpillars, 5th stage. Fed one meal of fresh pebrine material which had been dried under direct rays of sun for 2 days in a glass vessel. Fed on 22nd March, 1919.	18–2–19	27–2–19 5–3–19 12–3–19 20–3–19	29 3–19 and 30–3–19	9–4–19	Negative. 100 per cent. of the moths free from pebrine.	....
3	10 N. caterpillars, 5th stage. Fed at one meal leaf smeared with fresh pebrine material which had been dried in a glass vessel under direct sun's rays for 2 days, *viz.*, 29th and 30th June, 1919.	13–6–19	17–6–19 21–6–19 24–6–19 28–6–19	5–7–19	13–7–19	Negative. All the 10 moths free from pebrine.	The pebrine material used was found to be infective before drying in the sun. The control had 40 caterpillars. All found to be disease-free. It would be necessary to carry out further experiments to separate the thermal from the light effect.

O.

Experiments to see if a mildly pebrinized moth transmits pebrine.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	Progeny of a mildly diseased moth (spores of pebrine seen in the gut only of the moth).	21–9–16	25–9–16 29–9–16 1–10–16 4–10–16	10–10–16 and 11–10–16	21–10–16 and 22–10–16	13 caterpillars were examined on different dates, 2 showed pebrine on 11th October, 1916, for the first time. 2 pupæ were examined on 14th October, 1916, they were pebrinized. 100 per cent. of the moths were pebrinized.	....
2	Progeny of a diseased moth (pebrine found only in the gut of the mother moth).	27–6–16	30–6–16 3–7–16 6–7–16 10–7–16	15–7–16	27–7–16 and 28–7–16	52·7 per cent. of the moths showed pebrine.	....
3	Progeny of a mildly diseased moth (spores of pebrine seen in the gut only of the moth) (Hill rearing).	6–10–18	Not recorded	25–10–18 and 26–10–18	6–11–18 and 7–11–18	One moth out of 40 moths showed pebrine.	....
4	Progeny of diseased moths (which as larvæ were given one pebrinized meal one day before spinning).	22–7–18	25–7–18 28–7–18 31–7–18 3–8–18	11–8–18	....	100 per cent. of the caterpillars died in the pupal stage.	....

P.

Experiments carried out to see the effect of rearing silkworms at a high altitude (Shillong 5,000 ft.) for one generation.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	30 N caterpillars hatched out and reared at Shillong. When in last stage just a day before spinning were fed one meal infected with fresh pebrine on 7th September, 1918.	....	....	8-9-18	20-9-18	40 per cent. of moths showed very mild attack of pebrine. Eggs laid by these diseased moths were reared at Pusa. They practically did not show any disease. (1 moth out of 40 showed pebrine.)	The control had 90 caterpillars. All found free from pebrine but 3 pupæ suffered from fungus disease.
2	30 N. caterpillars hatched out and reared at Shillong. When in last stage just a day before spinning, fed two meals infected with pebrine material which had been kept dry in a desiccator from 15th July to 25th August, 1918. Infected meals given to the caterpillars on 7th September, 1918.	....	....	8-9-18	20-9-18	Negative. 100 per cent. of the moths were free from pebrine.	The control had 90 caterpillars. All found to be free from pebrine but 2 pupæ suffered from some fungus disease.
3	30 N. caterpillars, hatched out and reared at Shillong. Fourth stage. Fed on fresh pebrine material four meals, once a day, from 7th to 10th September, 1918.	....	3rd 7-9-18 4th 18-9-18	1-10-18	19-10-18	94 per cent. of the moths showed pebrine. Eggs laid by the diseased moths were reared at Pusa. They showed pebrine infection.	The control had 90 caterpillars. All found to be free from pebrine.

4	20 N. caterpillars, 3rd generation of those silkworms th t had spent one life-cycle at Shillong. Reared at Pusa. These caterpillars were kept after 2nd moult and reared till maturity in an infected cage which was covered with numerous fresh pebrine spores.	16-11-18	23-11-18 29-11-18 6-12-18 14-12-18	31-12-18	22-1-19	35 per cent. of the moths showed pebrine.	The control had 60 caterpillars. All found to be free from disease.
5	40 N. caterpillars, 3rd generation of those silkworms that had spent one life-cycle at Shillong. Reared at Pusa. Fed on fresh pebrine material at four meals after 3rd moult.	16-11-13	23-11-18 29-11-18 5-12-18 14-12-18	31-12-18	22-1-19	59 per cent of the moths showed pebrine.	The control had 60 caterpillars. All found to be free from pebrine.

Q.

Controls reared at Pusa (Gangetic Delta).

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	30 N. caterpillars reared at Pusa. When in last stage just a day before spinning were fed one meal of fresh pebrine material on 3rd July, 1918.	17–6–18	20–6–18 23–6–18 25–6–18 28–6–18	4–7–18	11–7–18 and 12–7–18	74 per cent. of the moths showed pebrine. Eggs of these diseased moths when reared showed pebrine.	The control had 60 caterpillars. All found to be free from pebrine.
2	20 N. caterpillars reared at Pusa, 5th stage. Given 2 infected meals of pebrine material which had been kept dry in a desiccator from 15th April to 23rd May, 1919. One infected meal given on 23rd May and another on 25th May, 1919.	29–4–19	4–5–19 9–5–19 13–5–19 17–5–19	27–5–19	5–6–19 and 6–6–19	30 per cent. of moths showed pebrine.	The control had 60 caterpillars. All found to be free from pebrine when examined as moths.
3	30 N. caterpillars reared at Pusa. Fourth stage. Fed two meals of fresh pebrine material on 6th and 7th June, 1918.	28–5–18	31–5–18 3–6–18 6–6–18 10–6–18	17th to 20th June 1918	....	100 per cent. caterpillars and pupæ that were examined were pebrinized. No moths emerged.	The control had 90 caterpillars. All disease-free.
4	20 N. caterpillars reared at Pusa, 4th stage. These caterpillars were kept in an infected cage which was covered with numerous fresh pebrine spores, after third moult, and reared till their maturity in the cage.	8–11–18	16–11–18 22–11–18 28–11–18 5–12–18	23–12–18	21–1–19	44 per cent. of the moths showed pebrine.	The control had 60 caterpillars. All found to be free from pebrine.
5	40 N. caterpillars reared at Pusa, 5th stage. Fed on fresh pebrine material, four meals after fourth moult.	8–11–18	16–11–18 22–11–18 28–11–18 4–12–18	23–12–18	22–1–19 and 23–1–19	100 per cent. of the moths showed pebrine.	The control had 60 caterpillars. All found to be free from pebrine.

Experiments to determine the least number of spores that would be sufficient to bring about infection.

No.	Description of experiment	Date of hatching of caterpillars	Dates of passing of the four moults	Date of spinning of cocoons	Date of emergence of moth	Results	Remarks
1	One N. caterpillar, 5th stage. Given a piece of leaf (20 mm. × 5 mm.) smeared with fresh pebrine material. Inoculum showing 5 spores in a field: objective $\frac{1}{6}$th × 10 ocular. The caterpillar ate half the piece of leaf on 24th June, 1919.	9-6-19	12-6-19 16-6-19 19-6-19 23-6-19	30-6-19	9-7-19	Negative ..	The control had 60 caterpillars. All found to be disease-free. Inoculum spread on leaf contained about 100 spores per sq. mm. of leaf surface = 5,000 spores ingested.
2	One N. caterpillar, 5th stage. Given a piece of leaf (40 mm. × 5 mm.) smeared with fresh pebrine material. Inoculum showing 5 spores in a field: objective $\frac{1}{6}$th × 10 ocular. The caterpillar ate the whole piece on 26th June, 1919.	9-6-19	12-6-19 16-6-19 19-6-19 23-6-19	30-6-19	9-7-19	Positive	Ditto but 20,000 spores ingested.
3	One N. caterpillar, 5th stage. Given a piece of leaf (10 mm. × 5 mm.) smeared with fresh pebrine material. Inoculum showing 5 spores in a field: objective $\frac{1}{6}$th × 10 ocular. The caterpillar ate the whole piece on 26th June, 1919.	9-6-19	12-6-19 16-6-19 19-6-19 23-6-19	30-6-19	9-7-19	Ditto	Ditto
4	One N. caterpillar, 5th stage. Given a piece of leaf (10 mm. × 5 mm.) smeared with fresh pebrine material. Inoculum showing 5 spores in a field: objective $\frac{1}{6}$th × 10 ocular. The caterpillar ate about $\frac{3}{4}$ of the leaf on 26th June, 1919.	9-6-19	12-6-19 16-6-19 19-6-19 23-6-19	30-6-19	9-7-19	Negative	Ditto

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